Oct. 1926. G. J. Ward.

LECTURES ON

NUTRITION

A SERIES OF LECTURES GIVEN AT THE MAYO FOUNDATION AND THE UNIVERSITIES OF WISCONSIN, MINNESOTA, NEBRASKA, IOWA, AND WASHINGTON (ST. LOUIS)

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INTRODUCTION

The lectures on nutrition herewith presented to the public were given during the year 1924–1925 under the auspices of the Mayo Foundation and the local chapter of Sigma Xi at Rochester, Minnesota; the Medical School of the University of Wisconsin at Madison, Wisconsin; the Graduate School of the University of Minnesota at Minneapolis, Minnesota; the Medical School of the University of Nebraska at Omaha, Nebraska; the Medical School of Washington University at St. Louis, Missouri; the Graduate School of the University of Iowa at Iowa City, Iowa, and the Des Moines Academy of Medicine at Des Moines, Iowa.

The lectures include a large portion of the recent research work in the field of nutrition. The lecturers were the persons who had conducted or been responsible in large measure for the several researches. The volume therefore is a statement by competent authorities of our present-day knowledge of most of the important problems of nutrition. While the lectures do not attempt to cover in detail the entire subject of nutrition, they do contain a large body of fresh information on the subject which it is believed will prove of live interest to the public.

Louis B. Wilson, M. D.
Director
The Mayo Foundation for
Medical Education and Research.

Rochester, Minnesota.
November, 1925.

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LECTURES ON NUTRITION

THE MEASUREMENT AND SIGNIFICANCE OF BASAL METABOLISM

FRANCIS GANO BENEDICT

As a result of the war the American people have become familiar with the word "calorie," and have begun to think of foods in terms of heat units. The word "vitamin" (that elusive but necessary food-accessory factor) has also appeared in the vocabulary pertaining to nutrition, and now the word "metabolism" bids fair to be firmly established therein. A calorie is a heat unit easily definable, if not easily comprehensible. Vitamins are things as yet unseen or at least seen but evanescently. Metabolism is a deep-seated life process, the products of which are in part visible, such as fat deposits in the body, and in large part invisible, as in the products of combustion.

DEFINITION OF METABOLISM

In spite of its complexity of meaning, the word "metabolism" is now in current use. Studies and measurements of metabolism are being made, and we hope that this process is being better understood. In its full meaning metabolism indicates the changes, constructive and destructive, which

take place in a living organism. In plants, obviously, the process is chiefly a constructive one, that is, the synthesizing of the carbohydrate of the cellulose and starch cells from the carbon dioxid of the air and water. In the animal those forces in metabolism producing growth represent the constructive phase, while the ordinary disintegration during the life processes represents the destructive phase.

The word "metabolism" formerly had a wide significance, for it included all transformations of matter and energy in

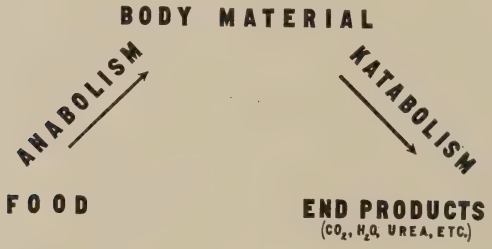

Fig. 1.

the body, that is, the processes of synthesis and of analysis. A simple chart helps to visualize these processes.

From this chart it is obvious that the constructive forces by which food materials are used to build up the body represent the anabolic changes, while the ordinary life processes, particularly when food is not given, are the katabolic processes. It is extremely difficult to make any real quantitative estimates of the processes of anabolism. By careful analyses of both income and outgo it is possible to find out if there has

been at any time a preponderance of anabolic changes, that is, if there has been a gain of chemical elements to the organism. This gain, of course, takes place chiefly during the period of growth. But such a measurement must of necessity in most cases be based simply upon a differential method. If the intake is larger than the output, the difference is considered to have been retained by the body. But to determine the exact course of any given food molecule in its transformation into body substance, and particularly to attempt to localize such a transformation, is beyond present-day technic. Anabolic processes have, therefore, been but little studied. If in a long period of excess feeding with carbohydrates a pig or a goose is fattened, we properly speak of a transformation of carbohydrate to fat. With human beings, a great many women and not a few men over forty know that such transformations are only too possible. But quantitative measures of such processes are practically unknown.

Strictly speaking, the transformations of any compounds, organic or inorganic, in the body are processes of metabolism, and since the nitrogenous transformations can all be measured by determining the nitrogen of income and the nitrogen of output (that is, the nitrogen in urine and feces) we often speak of "nitrogenous metabolism." The salts of calcium and magnesium and the compounds of phosphorus and sulphur are now regularly studied and reports are published on the metabolism of these elements. Yet the new trend, certainly among the laity and those whose friends have been in metabolism clinics, is to limit the meaning of the word to the metabolism of energy, especially as represented

by the gaseous exchange. Hence the application of the word "metabolism" is being more and more restricted to gaseous metabolism.

While the current meaning of metabolism excludes constructive or synthetic processes, since there is little or no heat involved in such processes, and excludes fermentations which involve but little heat, or enzyme action with its slight heat production, it does include those extensive breaking-down processes or oxidations accompanied by the production of large quantities of heat. In clinical medicine, the term "metabolism" and especially "basal metabolism" is today for the most part restricted to basal *katabolism* or the breaking down of body materials (chiefly fat and carbohydrate) by the processes of oxidation, whereby substantial amounts of heat are produced.

FACTORS INVOLVED IN METABOLISM

Clearly recognizing, therefore, that by metabolism we mean basal *katabolism* and that we are interested in the *katabolic* and not the anabolic processes, we may proceed to consider the factors involved in metabolism.

INGESTION OF FOOD

When food is eaten, both processes, anabolism and katabolism, are at work. In studying the transformations of matter and energy, however (which are in large part, although not exclusively *katabolic*), it is highly desirable and fortunately very practical to minimize, if not altogether to rule out, anabolism by completely withholding food. Even

with food withdrawal, when katabolic processes preponderate, there may be anabolic changes, for there are well-known instances of fish which not only live for a long time without food, but actually undergo great anatomic changes as a result of anabolic processes when fasting. The salmon enters the river from the sea with well-developed muscles, and proceeds up the river to the spawning grounds. While in transit no food is taken and the sexual organs become greatly enlarged by the transportation of nitrogenous material from the muscles, which become correspondingly reduced.

With all living organisms in the early period following food withdrawal, while there is still unabsorbed and undigested food in the alimentary tract, the anabolism may still be going on, indeed until the content of the alimentary tract no longer yields material to the blood stream. In the case of man, with small contents in the digestive tract, this period is relatively short, at the most twenty-four hours. A ruminant, such as the ox, however, has a large intestinal fill, amounting in a well-fed ox to one-fifth of the total body weight; and this mass furnishes at least a portion of the energy for life for from four to five days after withdrawal of food.

At the New York Zoölogical Park Mr. E. L. Fox of the Nutrition Laboratory staff has found that with large snakes the period of digestion may easily be prolonged a week or more by keeping them at a temperature below 20° C.

In any consideration of basal metabolism, therefore, it is most important to take into consideration the influence of food, for the ingestion of food causes an increase in metab-

olism; and although anabolic processes predominate, there is always a stimulus to katabolism and increased heat production.

DIFFERENCES IN INDIVIDUALS

That the ingestion of food increases circulation and gives a feeling of warmth is noted by everyone. There, is therefore, no question but what there is increased functional activity as a result of the ingestion of food. But not all factors which might affect metabolism are so easily recognized. Indeed, the heat production of human beings varies greatly with different individuals, and in the same organism the heat output is extremely variable. It is important, therefore, to attempt to catalogue those factors known to influence metabolism, and we may first consider the differences between different organisms, such as between men and boys, or women and girls.

It is obvious that a large organism will produce more heat than a smaller organism, and yet we are immediately confronted with the problem of the units of measurement to be employed in the comparison. Size may be indicated by at least three factors, weight, height, or the more commonly used surface area. The difficulty of isolating the effect of each of these three factors on metabolism can hardly be overemphasized. An analysis of the factors going to make up weight alone shows that we have to deal with skeleton, muscular mass, and fat. The oxidative processes are in large part centered in the muscular mass, and we have every reason to believe that body fat is more or less inert, so far as oxidations are concerned. Thus, a weight made up in

large part of body fat would be, so to speak, diluted with an inert material.

The exact valuation of the factors of weight, height, and surface area is best made by a most careful biometric analysis of a large number of metabolism measurements with individuals of varying sizes. From this biometric analysis[11] it seems clearly established that weight and height are both independent factors. With regard to surface area a great deal of discussion has been raised. In general it is perfectly proper to state that those persons having larger surface areas will have a larger heat output. Indeed, it is stoutly maintained by most physiologists that the heat production per square meter of surface area is constant for all warm-blooded animals, whether we are dealing with a man, horse, dog, or mouse. The Nutrition Laboratory's experience does not support this view.

Entirely aside from these anatomical factors of weight, height, and surface area, it has been definitely proved that age, independent of size, is also a factor which influences metabolism. Thus, the older the person, the less the amount of heat produced. In addition, sex has a definite influence, for women and girls have a considerably lower heat production than men and boys of the same size.

MUSCULAR ACTIVITY

Aside from the foregoing factors affecting metabolism in *different* organisms, there are others which affect metabolism even in the *same organism* where weight, height, age, sex, surface area, and muscular mass remain constant.

Consequently these factors must be recognized in any consideration of basal metabolism.

Muscular activity is the most important of these, for the smallest muscular motions increase heat production. Severe muscular work has a very great influence. Thus a man working to the limit of human endurance can for some time increase his heat production tenfold. The pronounced after-effects of work, especially of severe work, should also be considered. The work of walking up stairs, for example, has an after-effect which increases the metabolism for some time, and it is not until the person has lain down for at least one-half hour that this after-effect disappears.

ENVIRONMENTAL TEMPERATURE

Environmental temperature under ordinary conditions of laboratory measurement does not play a great rôle, but experiments in which there have been large temperature changes, particularly experiments with animals, have shown that the lower the environmental temperature the greater the heat production.

OTHER FACTORS

Psychic disturbances are accompanied by increased metabolism. Hence calm, repose, and quiet are essential.

Any fever is accompanied by increased heat production, for as the cells are warmed there is greater metabolic activity.

Sleep lowers metabolism, and is as yet a factor too little reckoned with.

State of nutrition is another factor to be considered. Changes in weight may be due, in the child, to natural processes of

growth, that is, the formation of flesh, fat, and skeleton. Changes in weight may also be due to loss of flesh or disturbances of balance between flesh and fat. A well-nourished person has a higher metabolism than one who is poorly nourished. A loss of weight of 10 per cent may be accompanied by a decrease in metabolism of 20 per cent.

HEAT PRODUCTION AND HEAT LOSS

Thus we see that the *fires of life* (as recorded by the measurements of the calorimeter or by the analyses of the exhaled

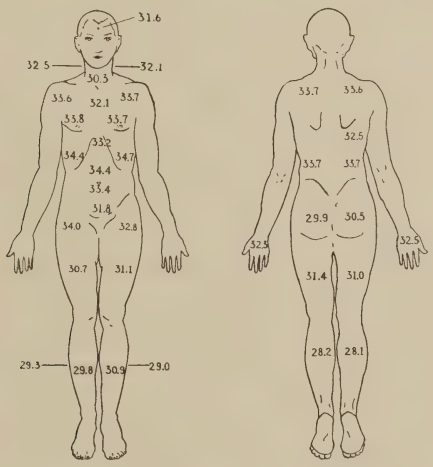

Fig. 2.—Skin temperatures at different parts of the body, under clothing.

air) burn with varying intensity and are subject to many influences. The measurement of the intensity of combustion by direct calorimetry deals only with heat output or heat given off by the body, and not with heat production. Heat production and heat loss are two very different things. Heat *production* is relatively stable under conditions of normal

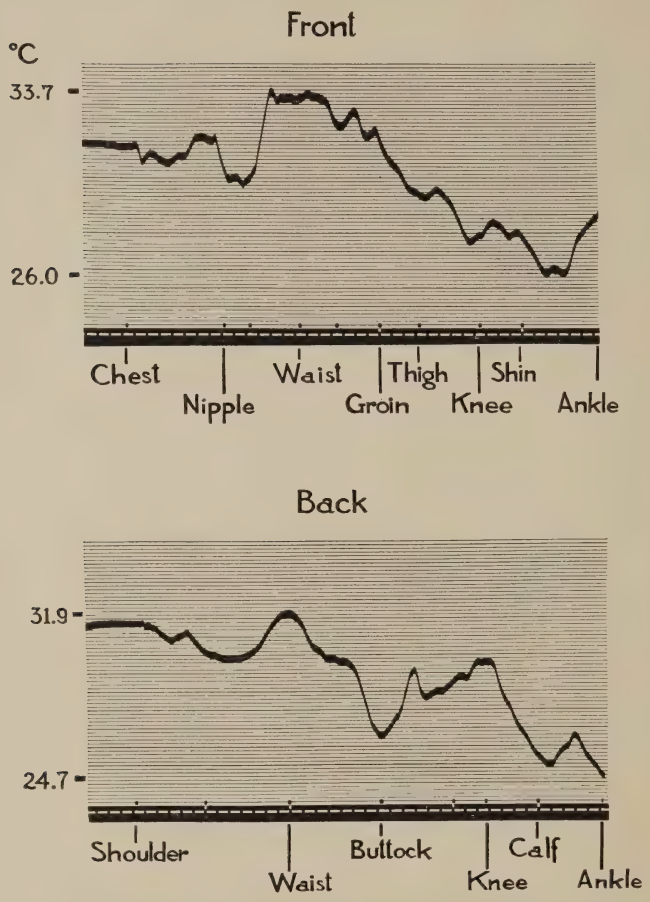

Fig. 3.—Photographic records from a string galvanometer for skin temperatures at different parts of the nude body following a one-minute exposure to an environmental temperature of 14.6° C. The thermal junction was moved at constant tempo from shoulder to ankle. Time registered at bottom of each curve is in two-second intervals.

repose, that is, there are no sudden changes unless one has muscular activity to deal with.

Heat *loss* to the environment is a very labile thing. The warm, living body has a normal internal temperature of 37° C. and a peripheral or skin temperature under the clothing, at different parts of the body, varying from 28° to 35° C. The environmental temperature may be very low, and the heat loss to the environment will naturally alter with the varying temperature gradients between the skin temperature and the environmental temperature. The flow of blood to the skin will alter the skin temperature and hence the rate of heat loss. The taking off of protective coverings such as gloves, hat, coat, or shawl, or the opening of an overcoat will instantly accelerate heat loss.

The temperature of the skin is variable, depending in large part on the rate of heat loss and much less on the heat production. Even under the clothing of a comfortably dressed person the skin temperature is by no means uniform (Fig. 2).

If the body is exposed, nude, to a room air of varying temperature, there are profound alterations in heat loss, as shown by the fall in skin temperature.

In Figure 3 the curve was made by connecting the thermojunction with an Einthoven galvanometer and drawing the junction slowly down over the right mammillary line. Before exposure the temperature of the trunk is more nearly uniform, and the effect of exposure of but one minute is clearly seen.

DIRECT AND INDIRECT CALORIMETRY

The course of the heat production under these conditions can be studied only by indirect calorimetry, that is, by measuring the oxygen intake and the carbon-dioxide output.

Innumerable tests show that with prolonged exposure there is an increase in the heat production, but it is not at all of the order of magnitude of the heat loss. Thus, with exposure to cold there is a great loss of heat and a slow, small increase in heat production. The body as a whole, therefore, has lost previously stored heat. This is proved by placing a thermometer between the clasped hands, or between the crossed arms, or the crossed legs, when a low temperature is found not only on the skin, but for the deep tissues, which have lost previously stored heat. This loss of heat (which could be measured in a calorimeter) is made up probably only in small part by an increased heat production.

Basal metabolism is not concerned primarily with labile heat loss, whether by radiation from the skin or vaporization of water from the lungs and skin; it is concerned with heat production.

Practically, therefore, basal metabolism cannot, in human beings, be advantageously determined by direct calorimetry, unless the experimental periods are long, and many persons find it difficult to keep still long enough. Fortunately heat production is easily measured by indirect calorimetry, that is, by the measurement of oxygen consumption and carbon-dioxide production. In ten minutes, by modern methods, a measurement may be made and the result calculated.

REPRODUCIBLE EXPERIMENTAL CONDITIONS

The methods of indirect calorimetry (and they are legion) are satisfactory only if strictest attention is paid to the fundamental, standard conditions under which such measurements

should be made. Physiologists, knowing the profound influence of certain factors on the heat production, have stipulated that for such measurements the subject should be in complete muscular repose and in the post-absorptive condition, that is, at least twelve hours must have elapsed since the last meal.

The modern scientist must furthermore anticipate, if possible, the existence of other factors, the importance of which will soon be recognized. Such foresight is essential, if the results of present tests are not to be rendered valueless in a decade because they were obtained without consideration of the influence of factors as yet perhaps unlisted.

MUSCULAR WORK OR REPOSE

The factor which most profoundly and most promptly affects basal metabolism is muscular work. After severe muscular work metabolism may be increased 1,000 per cent within two minutes.

Theoretically, every muscular movement is accompanied by heat production. Hence, theoretically at least, there should be complete muscular repose in all metabolism experiments. Certainly obvious voluntary muscular movements should be repressed. But the question arises as to how absolute this repression should be. So strong has this insistence on muscular repose become that conscientious observers reject experiments contaminated by visible activity, and so rigidly enforce inactivity that the muscular restraint becomes positively painful, if not unbearable, to many subjects.

MINOR MUSCULAR MOVEMENTS

Lefèvre,[13] of Paris, has criticized the emphasis which has been laid on the importance of muscular repose. By calculating the actual foot pounds required to raise the hand to the head, he shows that such a movement involves an insignificant amount of muscular work. To put the matter to test, a series of experiments were made by Mrs. Cornelia Golay Benedict and myself in the Nutrition Laboratory. With an especially quiet and well-trained subject the basal metabolism was first determined in complete repose, and then during a simple arm movement, such as raising the hand to the forehead every four seconds, that is, fifteen "silent salutes" per minute. The actual increase in metabolism (Table 1) proved to be but slight (as Lefèvre predicted), 1.5 c.c. of oxygen for each movement of the hand.

If such a movement of the hand once in ten minutes or even once a minute is negligible, one can fairly ask how a gross movement of the legs should be considered. To test this point the subject, while lying quietly, was asked to cross and uncross the legs once every twenty seconds. This, as well as the raising of the hand, was done to the beat of a metronome. The observations indicated that one such leg movement a minute would noticeably raise the basal metabolism. One movement in ten minutes, however, would be without significance.

While, therefore, isolated minor muscular movements, such as moving the hand to the head and back, have no influence on basal metabolism, movement of the legs must be proscribed. If the stringent rules of repose are to be relaxed

TABLE 1

INFLUENCE ON THE OXYGEN CONSUMPTION OF SMALL MUSCULAR MOVEMENTS OF ARMS AND LEGS

Subject A.			Subject B.		
Date and condition,* 1924.	Period.	Oxygen consumption per minute, c.c.	Date and condition,* 1924.	Period.	Oxygen consumption per minute, c.c.
January 3:			February 29:		
Basal	I	193	Basal	I	256
Basal	II	188	Basal	II	255
Arm movement	III	218	Basal	III	253
Basal	IV	195	Arm movement	IV	280
January 7:			March 1:		
Basal	I	200	Basal	I	257
Basal	II	196	Basal	II	255
Arm movement	III	210	Basal	III	244
Basal	IV	193	Arm movement	IV	268
Leg movement	V	222	Leg movement	V	285
January 9:					
Basal	I	203			
Basal	II	189			
Arm movement	III	224			
Leg movement	IV	209			
Basal	V	189			
January 16:					
Basal	I	202			
Basal	II	195			
Arm movement	III	215			
Leg movement	IV	225			
Basal	V	200			

*In basal periods subjects were lying, clothed, and covered with light blanket. In the arm movements the hand was raised to the forehead every four seconds. In the leg movements the feet were crossed every twenty seconds.

a particle (and it is certain that with some persons they should be somewhat relaxed) one always runs the danger of letting the bars down too much. A good rule is to insist on repose, complete if possible, but not such as to result in tension or distress, for moderate repose is far better than a tense or cramped position. Muscular activity greater than slight, visible muscular actions justifies ruling out the period of measurement, and it should be ruled out before the analysis or computation is made.

INFLUENCE OF BODY POSITION

Since the lying position is commonly believed to be more conducive to complete relaxation than the sitting position, metabolism measurements are usually made with the subject lying. A study has recently been carried out by Mrs. Benedict and myself on the influence of posture on metabolism,[4] and we have added our data to the somewhat extended earlier data. With a trained artist's model the metabolism was determined in the three different positions. The comparison between lying and sitting is shown in Table 2.

TABLE 2

COMPARISON OF THE OXYGEN CONSUMPTION PER MINUTE IN THE LYING AND SITTING POSITIONS.

Date.	Lying (awake), c.c.	Sitting, c.c.
December 1, 1920	180	191
December 3, 1920	173	186
December 6, 1920	180	188
December 17, 1920	188	196
January 19, 1921	195	216
January 26, 1921	188	195
January 27, 1921	187	192
November 3, 1921	194	193
November 4, 1921	200	204
November 5, 1921	199	203
November 7, 1921	207	215
November 10, 1921	202	208
February 8, 1924	188	194

From these data it is seen that with complete repose the effort of comfortably sitting in a chair is scarcely greater than that of lying on a couch. From these observations as to the degree of repose and the position of the body, we can say that for basal metabolism measurements the patient

may either lie or sit in a steamer chair, well supported, so that there is no strain. Relaxation is the keynote. The aim is not "rigid" repose, but "inert" relaxation.

PRECEDING MUSCULAR ACTIVITY

For basal metabolism work it is also very important to recognize and attempt to minimize the effect of muscular activity preceding the measurement. The subject must not run or walk fast in the open air, and must not walk up several flights of stairs prior to the test, for muscular work has a pronounced after-effect. Indeed, we are beginning to suspect that the effort of walking on icy sidewalks or against a severe wind, shivering, and exposure to cold prior to metabolism tests are all reflected in the subsequent metabolism measurements.

FOOD INGESTION

Muscular activity, then, is the factor which affects metabolism most profoundly, but food ingestion with the resultant digestive activity is also an important factor.

The fact of an increased heat production after food ingestion has been known since the days of Lavoisier and Séguin, but it is due to the work of Zuntz and especially Rubner that an explanation of this increase was first offered. Rubner stated that the rise was caused by the specific dynamic action of the foodstuffs, thus designating the cause, but not really explaining it. Zuntz worked with large domestic animals, ruminants. The content of the intestinal tract of these animals is very bulky, fibrous food which remains a long time in the intestinal tract and which must be worked over

and over, pushed along the tract, and the undigested portions finally expelled in large fecal masses.

Rubner found protein to have the largest specific dynamic action, but Zuntz, seeing that with practically protein-free rations there was, with ruminants, an enormous increase in heat output, concluded that this increase must be due to something other than protein. Zuntz thought of the seemingly large amount of work performed. Later, American experiments showed that the use of purgatives or of agar-agar, producing a rapid succession of stools, was without effect on the basal metabolism of man. Dogs, whose pancreas had been in large part extirpated and in consequence had a very low absorption for protein and fat (resulting in voluminous stools) showed practically no specific dynamic action when fed meat rations. Consequently, it was hardly possible to conceive of the mechanical work of peristalsis and evacuation as the cause of the increased heat.

Many years ago Friedrich Müller suggested, in a rather remote publication,[15] the possibility of the increase being due to cell stimulus. From various reasonings it appeared that acids such as the amino-acid in the protein and fatty acid of acidosis or partly burned fat were the cause of the increased heat, inasmuch as they produced a stimulus to the body. Thus with the acidosis of diabetes, for example, there is increased metabolism. When levulose is fed, there is strong evidence that it is converted into fatty acids. This explains, at least in large part, the pronounced stimulating effect of levulose ingestion.

With cane sugar, consisting of half levulose and half dex-

trose, the increment is somewhat less. Finally, the large increase found with ruminants may be the result of the extensive fermentations in the intestinal tract. Sixty years ago Herbert Grouven[9] in Salzmünde proposed the theory that with ruminants all carbohydrates were absorbed not directly into the blood stream, but through fermentation and through the path of fatty acids. With ruminants an increase in the heat production with a non-protein ration is a fact. Mechanical movements can hardly account for it, and the theory of acid stimulation is probably the best explanation, since the formation of fatty acids by the fermentation of carbohydrates would result in their being absorbed and being carried to the cells, and there acting as a stimulus.

PERMISSIBLE BREAKFAST PRIOR TO METABOLISM TESTS

Under all these circumstances it is seen that the taking of any food (particularly protein and ketose sugars) prior to basal metabolism tests should be proscribed. This is the common practice at present, but there are two rather serious objections to it. In the first place, many people believe that going without food, even for one meal, is harmful and weakening. Hence there is often a real or fancied feeling of faintness and dizziness, which increases the apprehension felt by the novice in the first metabolism test. In the second place, the complete withdrawal of food may, with children and also with the obese, soon bring about an acidosis which, as we have seen, stimulates metabolism, so that the induced acidosis might increase metabolism more than the effect of the food.

For these reasons there is a certain modern tendency to consider the advisability of allowing a small, non-stimulating breakfast. Du Bois[6] permitted a small breakfast to secure a feeling of satiety and repose in some very active boy scouts, whose basal metabolism he wished to measure, uncomplicated by restlessness.

At the Nutrition Laboratory, capitalizing later knowledge regarding the effect of special foods, Mrs. Benedict and I proposed a breakfast[3] (Table 3) characterized by the absence of protein and the ketose sugars and by the presence of fat, which gives a greater feeling of satiety. Such a meal

TABLE 3

NUTRITION LABORATORY PERMISSIBLE BREAKFAST

1 cup (200 c.c.) of caffeine-free coffee.
16 milligrams of saccharin.
30 grams of medium cream.
25 grams of potato chips.

Total calorific value about 250 calories.

has no appreciable effect on metabolism, "stays by" the person, and gives a feeling of euphoria that is not purchased at the expense of a metabolism stimulated by protein or ketose sugars. All technicians find that the most difficult day for the new patient is the first day of the tests. It would seem as if the apprehension of the first day could be lessened if this light breakfast were allowed. The test could then be repeated on the next day, with the subject in the *post-absorptive condition*, that is, at least twelve hours after the last meal, which should not have contained a large amount of protein.

SPECIAL FACTORS

There are several factors which, although supposed to have an effect on basal metabolism when brought to play intensively on the human organism, may not in moderate degree prove to be sufficiently effective to demand special attention.

ENVIRONMENTAL TEMPERATURE

The effect of a cold environment is the first of these factors. If a person is exposed to cold so that he shivers, the metabolism must be greatly raised, for shivering is a muscular act, and as we have already seen, muscular work has a profound effect. Exposure to cold results in loss of body heat and a disturbance in the storage of heat. Soon the shivering starts up and the heat production now attempts to keep pace with the heat loss. But in a sense this is an indirect effect of the cold on heat production through the muscular work of shivering. Does the cold per se, independent of shivering, affect basal metabolism? This question is still much discussed. The Nutrition Laboratory's data, which are rather extensive, point to a certain production of heat without shivering, as incident to prolonged exposure to cold. Rubner has long maintained that there is a specific heat production as a result of exposure to cold. On the other hand, Johansson in Stockholm and Loewy in Berlin have claimed that there is no excess heat production without shivering. Johansson's experimental data are flawless, and no experimenter has ever secured a greater degree of muscular repose than he. Under these conditions he finds no effect of cold, even on the nude body.

If the body, as a whole, is exposed to cold air, heat is lost to the air. Thus, the face, hands, and neck lose heat. But what is the effect on the heat production? There are two possible means of securing temperature equilibrium: (1) By decrease in the blood supply to the exposed parts, until the temperature of the skin becomes lowered so that the normal heat loss is again attained, and (2) by increased heat production to compensate for the increase in heat loss.

In the latter case the skin temperature tends to remain constant, and in the former it becomes lower.

To test these points experiments have been in progress at the Nutrition Laboratory for several years. A subject, capable of withstanding prolonged exposure to cold, of unusually placid temperament, and with extraordinary powers of relaxation and repose, was found in a very co-operative professional artist's model. When the subject disrobes, little or no change in the basal metabolism is found at first, thus confirming Johansson's short experiments. But as time goes on and the body and peripheral tissues become colder, there is a noticeable increase in metabolism long before shivering sets in.

The first and almost instantaneous effect of exposing the previously warm, clothed body to cold is a great wave of heat loss, due to the great temperature potential between the skin (approximately 33° C.) and the environmental air (approximately 15° C.). This pronounced heat loss is followed by a lowering of the skin temperature and, indeed, tissue temperature, thus reducing the temperature potential. Finally,

the skin temperature ceases to fall, and the heat loss adjusts itself to the prevailing temperature difference.

A calorimeter (a so-called "emission calorimeter") sufficiently sensitive to measure the actual heat loss in very short periods, of one minute or less, has only recently been available.

The subject was placed inside of the emission calorimeter, covered with several layers of blanket, but otherwise nude. The heat loss, which was compensated by an electric current of measurable intensity, was established under these conditions

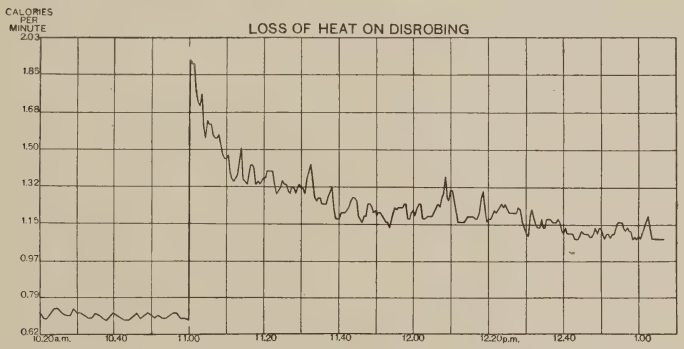

Fig. 4.—Comparison of loss of heat before and after disrobing. From 10:20 to 11:00 a.m. the subject was nude, but covered with blankets. At 11:00 a.m. the blankets were suddenly removed.

for approximately an hour or more. Then at a given instant, by means of previously arranged cords, the blankets were suddenly rolled back, thus producing the exposure of the nude body to the cold air. The heat lost as a result of this exposure is indicated by the pronounced rise in the curve. There is then a subsequent decrease in heat loss as the surface of the body cools off, until finally an approximate level is reached where the loss is fairly constant, but at a dis-

tinctly higher level than when the subject was lying, covered with a blanket.

A man lying uncovered on a hot, tropical night has a rectal temperature probably not much different from that of the arctic Eskimo in his ice-lined igloo, wrapped in furs. But in the case of the Eskimo certainly the exposed parts of the body, such as the face and hands (if not protected), must have a much lower temperature. Is the metabolism of the man in the tropics different from that of the man in the arctic or temperate zones? This question is still being stoutly debated.

Without considering these extremes of temperature, the question may properly be raised as to whether the environmental temperature should not be essentially the same in all metabolism measurements that are to be compared, that is, under conditions supposedly reproducible for basal metabolism measurements? No specific requirement for environmental temperature is included in the modern stipulations for basal conditions. It is the common custom, however, to make tests at "room temperature," presumably somewhere between 15° and 25° C. and it is tacitly understood by all workers that the subjects should be assured a comfortable temperature, neither too hot nor cold enough to induce shivering. Undoubtedly too little attention has been paid to this point heretofore, but many of the fundamental measurements of the metabolism of human beings have been made with respiration calorimeters which are so constructed mechanically that the environmental temperature is automatically held approximately at 20° C.

Little, if any, attention has been paid to publishing the

data regarding the kind and the amount of clothing and bed covering. For this neglect Lefèvre[12] has rightly criticized us. Indeed, Lefèvre goes so far as to maintain that, owing to the conditions for heat loss present in practically all basal metabolism measurements as at present made, the heat loss is so great that so-called "basal" metabolism, as measured, is always higher than the true basal. Measurement of the true basal is secured only when every precaution has been taken to prevent loss to the environment. This is possible, he maintains, only when the body is immersed in an indifferent water-bath at 35° to 36° C., for under all other conditions there is an excess heat production to combat the loss to the cold environment.

If Lefèvre's contention is true, then obviously current basal values are all too high. This concept constitutes a serious challenge to modern metabolism measurements. Although at the Nutrition Laboratory we were convinced that Lefèvre had fallen into the common error of so many critics of not clearly differentiating between heat loss and heat production, the criticism of modern metabolism technic seemed to us too plausible to go unchallenged. Accordingly, Mrs. Benedict and I have made a series of tests[4] with several subjects, in which the basal metabolism was first measured while the subjects were lying, clothed and lightly covered, in the laboratory, in a rather unusually low room temperature of 15° C., to accentuate, if possible, the influence of the cool environmental temperature. The subjects were clothed as ordinarily and covered with one thickness of a light cotton blanket. Immediately after the measurements

under these conditions the subjects entered a neutral bath (35° C.) in a very warm room (30° C.), and several metabolism experiments were made under these conditions.

Since all the subjects were well trained to metabolism measurements, the basal values found prior to the bath show

Fig. 5.—Measurement of the oxygen consumption of a subject during immersion in a water-bath.

reasonably close agreement with each other. After the subjects entered the bath the metabolism measurements, instead of decreasing, as predicted by Lefèvre, almost invariably tended to increase slightly. The results are shown in Tables 4 and 5.

TABLE 4

MR. B., AGE FORTY-SEVEN YEARS; NUDE WEIGHT, 67 KG.; HEIGHT, 169 CM.

(Oxygen consumption in cubic centimeters per minute)

Date.	Basal (room temperature 16° to 18° C.).			Bath at about 36° C.					
October 4th	204	205	197	208	215	210	202		
October 5th	203	207	200	211	222	223	224	230	
October 6th	196	220	213	212	213	215	211	236	224

TABLE 5

MISS W., AGE THIRTY-THREE YEARS; NUDE WEIGHT, 60 KG.; HEIGHT, 162 CM.

Date.	Room temperature, ° C.	Oxygen consumed per minute.					Temperature of bath, ° C.
		Basal (average), c.c.	Bath, c.c.				
December 7th	21.0	192	219	210	186		37.0
December 8th	15.5	190	194	186	191	190	36.3
December 10th	16.2	194	182	200	206	187	36.5
January 3d	15.7	192	195	195	197		35.0
January 24th	14.8	198	194	200	192	196	34.0
January 25th	14.8	198	208	201			33.2
January 28th	15.1	200	191	193	206		38.0

From these tests one can conclude that an environmental temperature of 15° C. or above, when the subject is clothed and lightly covered, does not raise the metabolism, or at least the metabolism is not lowered by subsequent complete immersion in a neutral bath of 35° to 36° C. Consequently a room temperature of 15° C. (if the subject does not feel cold and is protected from drafts) is a suitable thermal con-

dition for making basal metabolism measurements. Immersion in a water-bath at 35° C. is neither practical nor effective in lowering the metabolic rate.

FEVER

The important findings of Du Bois[7] on the influence of body temperature elevation on metabolism should not be disregarded. Measurement of the body temperature, preferably rectal, should assure the operator that a body temperature prevails within normal limits, taking into consideration the well-known diurnal temperature variations. Slight elevations in body temperature may make a metabolism measurement worthless for comparative purposes.

MENTAL ATTITUDE AND PYSCHIC REPOSE

While the two most pronounced factors affecting metabolism are food ingestion and muscular work, the factor of temperament or mental repose may be of significance, although it may be secondary, acting through the muscles. If the subject is nerved up, apprehensive, and irritable, this condition contributes to an increased metabolism. For example, one of our subjects came to the laboratory once seemingly very sleepy and exhausted. To our surprise his metabolism was high, but it was found that a conflict the night before with an irate father, with whose daughter he had tried to elope, had left a somewhat shattered nervous system with an increased metabolism that showed above the seeming muscular exhaustion and somnolence. On two instances also we have had to contend with the after-effects

of an alcoholic night with some of our subjects. Such orgies raised the metabolism.

Unquestionably apprehension and fear raise metabolism. Hence all methods to depress such apprehension are justifiable. As already pointed out, the steamer chair as a substitute for the bed and the permissible breakfast may both produce a feeling of euphoria, allay fear, and banish apprehension to such a degree as to warrant their intelligent use. The long wait prior to metabolism tests, the sight of seemingly complicated apparatus, the attachment of nosepieces, mouthpiece, or face mask, should all occur under the least irritating conditions and in the shortest time possible.

SLEEP

Under conditions of sleep one would expect to have the greatest degree of muscular and psychic repose. Hence the influence of sleep on metabolism possesses unusual interest for us. This problem is not a simple one to study, for with the onset of sleep coöperation on the part of the patient disappears, but also (it should be equally emphasized) antagonism disappears. Consequently experiments on the effect of sleep must, for the most part, be made in some form of respiration chamber where mouthpiece, nosepieces, or mask are not employed. With an especially well-trained subject it is not impossible to make such observations. The Nutrition Laboratory has certain data with regard to sleep, as yet incomplete, which justify us in stating that the influence of sleep is very pronounced on the mechanics of respiration, causing transitory and perhaps permanent changes

in ventilation rate and the storage of carbon dioxide in the body.

Whether sleep affects the total metabolism is by no means clearly established, although during sleep there is a distinct tendency towards a lower metabolic level. Hence for practical purposes and for reproducible conditions it is clear that

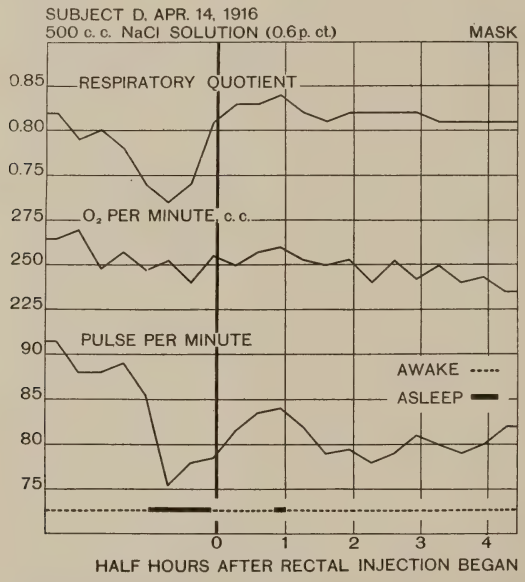

Fig. 6.—Respiratory quotient, oxygen absorption, and pulse rate of Subject D, April 14, 1916, before and after rectal injection of 500 c.c. 0.6 per cent. solution of sodium chloride.

metabolism measurements should only be made with the subject awake.

The curve in Figure 6 (one of many taken from a forthcoming monograph by my associate, Dr. T. M. Carpenter) shows two striking effects of sleepiness: first, the fall in

the pulse rate, and second, the profound disturbance in the respiratory quotient. The absence of sleep is insisted on to such an extent in the Nutrition Laboratory that Dr. Carpenter frequently arranges a signal magnet near the ear of the subject, which gives a slight sound at the end of each half minute, and the subject is provided with a push button to register his reaction to this. The absence of registration is an indication either of drowsiness or of complete sleep.

While it is true that most patients will be too alert psychically to sleep, not infrequently drowsiness does occur. In all such cases we believe it is necessary for the operators to insure that their patient is awake during respiration experiments. This is particularly the case if the respiratory quotient is to be studied.

CONDITIONS SPECIFIED FOR METABOLISM MEASUREMENTS

With a consideration of the foregoing factors, which are known or supposed to influence basal metabolism, we are in a position to specify perhaps more exactly than before the best and most practical reproducible conditions for measuring basal metabolism.

The first and foremost requisite is repose. The subject should be in the greatest degree of relaxation, either lying on a couch or in a steamer chair. The injunction for complete muscular repose should not be so strict, however, as to result in the person being cramped or muscle-bound. A graphic tracing of the activity, with the employment of a pneumograph either about the thighs or under the bed spring, is helpful.

The second requirement is the post-absorptive condition. No food should have been eaten for twelve hours and the last meal should not have been excessively high in protein. It is doubtful if a special diet for several days prior to the test is of real value. With the demonstrated absence of the stimulating effect of a permissible breakfast, such a light meal can be given, although at present it will be best to give it only on the first day of the test. If the second day's measurements are lower than the first, these values should be used, for in general it should be stated that, barring technical errors, the lowest values found represent basal metabolism and all other values are too high.

The subject should be comfortably clothed and not too warm or too cold. The amount of protection should be such as to secure the greatest degree of relaxation.

Every effort should be made to combat apprehension by avoiding an undesirably long wait prior to the tests and unnecessarily prolonged arrangement of apparatus, mask, mouthpiece, or nosepieces. A quiet, placid relaxation is ideal.

The body temperature (if feasible, the rectal temperature should be measured; if not, the buccal) should be within normal limits. If morning buccal temperatures are over 99° F., the time should not be wasted on a basal metabolism measurement.

The subject should be kept awake. A signal with a push button response is desirable, not only as a proof that the subject is awake, but because it is a good thing psychologically to have the patient's mind on some simple, non-muscular task.

STANDARDS OF BASAL METABOLISM

If the metabolism is measured under such stipulations, one can consider it either basal or at least standard, for the conditions are standardized and are reproducible. Measurements made under similar conditions are, moreover, logically comparable. Whether the measurements are truly basal or not may still be debated. Certainly with complete muscular and mental repose, without food in the stomach for at least twelve hours prior to the measurement, with a comfortable environmental temperature, without a febrile temperature, the only factors capable of lowering the metabolism under these conditions are deep sleep and prolonged fasting or undernutrition. Bearing in mind that the chief object in basal metabolism measurements is to secure reproducible, comparable conditions, it would seem as if the above specifications are all that should be insisted on at the present time.

SEASONAL VARIABILITY

Are the results obtained under these conditions comparable and are they comparable with the rather considerable number of basal metabolism measurements heretofore published? In other words, is the standard metabolism as measured constant? This question applies, first, to the individual. Since under the specified conditions of measurement the immediate factors influencing basal metabolism have been ruled out, the doubtful element is primarily only the matter of season. Measurements made at night on a group of young men inside of a respiration chamber at the Nutrition

Laboratory did show a tendency for a decrease in metabolism between the first of October and the first of January.[5] It is also possible that the same individual will have a very different metabolism in the arctic or temperate zones than he will after a sojourn of several months in the tropics, but this is not as yet definitely established.

For all practical purposes one can conclude that with the same individual the basal metabolism remains reasonably constant from day to day and even from season to season. That there are not profound influences due to considerable changes in habits of life is not as yet disproved. The laboratory dog of Lusk,[14] after a sojourn in the country, showed an increased metabolism of 20 per cent, although there was no change in weight. This fact suggests that the effect of recuperation after a summer's holiday is well worthy of further investigation.

COMPARISON OF THE METABOLISM BETWEEN INDIVIDUALS

It is of greatest importance to the clinician, however, to know whether the basal metabolism as measured on one individual may be compared intelligently with that measured on other individuals or groups of individuals. Is it not possible to find some method of intelligently comparing the measured metabolism of a patient with the general trend of metabolism of other individuals of similar age, height, weight, and sex? This has been attempted in several ways. One of the earliest methods was to compare the metabolism on the basis of the body weight, on the assumption that a large person would have a larger heat production than a smaller person.

Hence it was argued that the more rational method of comparison was on the basis of the heat production per kilogram of body weight. This comparison, however, is, at least with adults, fundamentally wrong, inasmuch as it assumes that each kilogram of body substance has exactly the same heat-producing power, whereas we know that inert fat is not comparable to active muscle as a producer of heat.

Another comparison which has had a most pronounced influence on the interpretation of metabolism measurements was that early recognized as the relationship between the heat lost from the surface of the body and the surface area. It was found, as would be expected, that the heat loss (about 1,000 calories for each square meter every twenty-four hours) was approximately proportional to the surface area, not only with different individuals of the same species and different size, but likewise between different species. The "surface area law," therefore, became one of the most important contributions to our knowledge of the physiology of energy transformations.

From the practical standpoint it is perhaps not a matter of importance whether the rate of metabolism is directly proportional to the surface area, whether it is controlled by the heat loss from the body, whether the heat loss is independent of the heat supply, or whether the heat produced is determined by the active mass of protoplasmic tissue and the stimulus to the cells. But it is important to know whether there is a referable basis which can be used intelligently for comparing various individuals.

At the outset it may be stated that the original surface

area law, as outlined by Rubner, implied that the metabolism was proportional to the surface area, and that this law was subsequently extended throughout almost the entire animal kingdom and has since been erroneously extended to cold-blooded animals. Its application to human beings has already been limited by common consent in two ways, in that an equal surface area is considered to have a different value between men and women and a different value between the old and the young.

The Nutrition Laboratory's data for children and adults are shown in Figure 7 on the basis of the heat production for each square meter of body surface every twenty-four hours. Here the influence of sex is shown throughout practically the entire life, except in the early months, and the influence of youth is evidenced by the extraordinarily low metabolism at birth or shortly after, with a maximal metabolism at about the age of one year.

CURRENT STANDARDS OF REFERENCE

There are in current use two standards of reference.* One is based directly on the surface area, making allowance for sex and age. This standard of reference is extremely simple and very practical, and the medical profession and physiologists as a whole owe a great debt to Dr. Eugene F. Du Bois[1] for it. The rule-of-thumb procedure of the earlier surface area estimations is avoided in this standard by including the remarkably accurate measures of the surface

* The standards of Dreyer (Lancet, 1920, Part 2, p. 290) are not here considered, although attention should be called to this work with its possible potentialities.

area of the human body introduced by Du Bois and Du Bois.[8] With such measured surface areas, or with surface areas

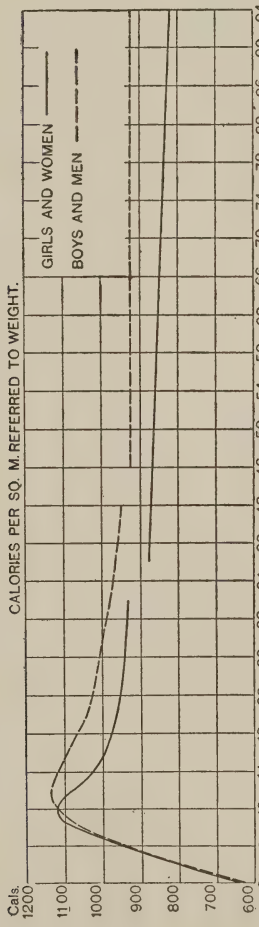

Fig. 7.—Comparison of the basal heat production of children and adults per square meter of body surface per twenty-four hours referred to weight.

derived from their simple formula, one can, by knowing the surface area, age, and sex of the individual, compare the

measured metabolism with a standard which is now extensively used, the so-called Du Bois standard.

The Nutrition Laboratory, believing, as a result of more intimate biometric analysis, that the factors affecting metabolism independently are sex, age, weight, and height, and dealing for the most part with physiologic rather than pathologic problems, has contended that a biometric formula involving these four factors is scientifically better founded. Hence we have the prediction formulas printed in collaboration with Professor J. Arthur Harris[10] of the University of Minnesota:

For men..... $h = +66.4730 + 13.7516w + 5.0033s - 6.7550a$
For women..... $h = +655.0955 + 9.5634w + 1.8496s - 4.6756a$

h = total heat production each twenty-four hours.
w = weight in kilograms.
s = stature in centimeters.
a = age in years.

Innumerable comparisons of these two methods of standardization have been made, but when one considers that both standards were based in large part on the same experimental material, that is, on the Nutrition Laboratory measurements, it is not surprising that they agree so well.

Any logical basis for the use of the surface area law in medicine is enormously complicated by the fact that the adherents of the surface area law stoutly maintain that it was never meant to apply to other than definite physiologic conditions. Yet they do not hesitate an instant to refer measurements in pathologic cases (in which obviously undernutrition and at times fasting necessarily occur) to normal

standards, and because they find the metabolism for each square meter of surface area is the same as the standard, they do not hesitate to pronounce it normal, overlooking entirely that a lowered metabolism due to undernutrition may be accompanied by a superimposed effect of some disease.

It is more than likely that the time is now at hand when definite quantitative attention must be paid to the state of nutrition of various patients, in interpreting their basal metabolism. The difficulty of securing any uniformity in the method of indicating the state of nutrition is very pronounced. The Nutrition Laboratory has been quite inclined to accept Pirquet's pelidisi as one of the best numerical

$$\text{Pelidisi of Pirquet} = \frac{\sqrt[3]{10 \times \text{weight (gm.)}}}{\text{sitting height (cm.)}}$$

indices thus far available, leaving to medical men the value of his subsidiary observations with regard to the turgor, amount of body fat, and blood supply to the skin.

In a recent analysis of the metabolism of a large number of girls and women it was found that when the measured twenty-four-hour heat production was divided by the pelidisi of these individuals, a straight line function was found, beginning with children as young as three months and extending to old age.[2] It would thus appear as if many of the aberrant deviations from the general trend of metabolism measurements, noted particularly with girls and women, disappear when the state of nutrition, as indicated by the pelidisi, is taken into consideration.

It is clear that the state of nutrition of the individual is pronouncedly reflected in the general metabolism. During complete fasting the metabolism is very low. During undernutrition it is likewise very low. With surfeit feeding it is

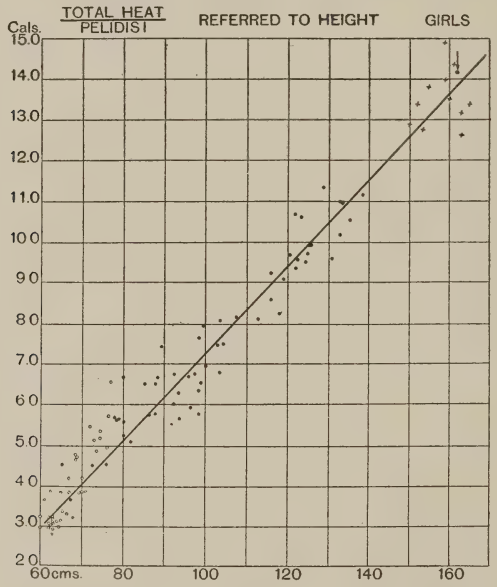

Fig. 8.—Relationship between height and the basal heat production each twenty-four hours divided by the pelidisi, with females from one week of age to full maturity. The hollow dots represent girls below 10 kilograms in weight or younger than one year; the solid dots represent girls 10 kilograms or above in weight; the crosses represent groups of girl scouts, twelve girls in each group; and the arrow indicates the average value for 103 adult women.

very high. All of these conditions are entirely distinct and separate from the immediate effects of food ingestion. The fact that so-called "hospital normals" have a low metabolism, the fact that Lusk's dog, when confined to the laboratory

for several months, had a low metabolism, the fact that a large group of students subsisting on half rations had a very low metabolism, coupled with the fact that by excessive feeding there may be an increased metabolism, such as to strengthen belief in Voit's old idea of "luxus consumption" (so strongly advocated at the present day by Grafe), are of themselves sufficiently important to indicate the basic significance of the "state of nutrition."

Basal metabolism is reasonably constant in the same individual. It is also reasonably comparable between different individuals, if weight, height, sex, and age are taken into consideration and provided that the pronounced influence of under- or overnutrition is borne in mind. It is not fixed or determined by surface area and the loss of heat to the environment. While undeniably in a state of flux and subject to the influence of other factors than those of age, size, and sex, it still remains a fact that basal metabolism (depending on heat production and not heat loss) is a very good index of the general state or level of vital activities as produced by the mass of the cells and the stimulus to their activities.

Modern, simplified methods make possible the determination of basal metabolism with a technic that is much easier than the technic for counting the blood corpuscles, and with an instrument that is not appreciably more expensive. In fact, it is not perhaps too speculative to state that the present wave of enthusiasm for basal metabolism measurements and their application primarily to endocrine disturbances may in time be replaced by a more rational use of this important

measurement as an index of general vigor, tone, and physiologic state.

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PROBLEMS OF METABOLISM

Graham Lusk

In 1870, fifty-five years ago, Carl Voit[6] wrote an article of a hundred pages entitled, "The development of the doctrine of the source of muscular energy and of the doctrines of nutrition during the past twenty-five years." He is resentful of the criticisms of Liebig, who was then sixty-seven years old and no longer actively experimenting. He points out that Liebig himself in 1844, twenty-six years before, had denounced Berzelius, who at that time happened also to be sixty-seven years old, on the ground that Berzelius had ceased to experiment, devoted his entire intellectual powers to theoretic speculations, and was therefore not competent to criticise the experimental work of others. Voit closes his paper with the following words:

"While preparing this defense of my viewpoint to which I have been driven, there has grown upon me the conviction that the knowledge of nutrition is in a state of unhindered and flourishing development. The ship which for a long time was only moved by cross currents to and fro, because it did not have the rudder called 'experiment,' now moves forward uninterruptedly on its course. Let those who desire to remain uncertainly adrift continue this attitude, but they will be outdistanced and they will have no part in the re-

ward when the other richly laden vessel returns to the home port."

Such words clearly emphasize that only by the experimental method can one hope for progress and success. Also, one of the fundamental necessities in all scientific work is to know the charted sea. I would suggest a better knowledge of the old-time literature. Last year I gave to Robert Weiss, a pupil of Biedl's, an article written by Voit, but published in my name in the Zeitschrift für Biologie, vol. 27, 1890, entitled "Ueber den Einfluss der Kohlehydrate auf den Eiweisszerfall." Weiss returned the volume to me with the remark, "It is all absolutely modern." It seems to me that perhaps the only advantage of advancing age is that one is able to recall what the old masters thought. One has but to go back to the work of Liebig to find ideas that one naturally attributes to Rubner, or to read the work of the early French school of eighty to a hundred years ago to see where Liebig received his inspiration. When Lafayette Mendel and I were young men together in New Haven I called his attention to Voit's "Stoffwechsel und Ernährung," which he has since read many times and which still deserves attention, not only from us in America but also from many in Germany where it is well-nigh forgotten. Modern workers are not sufficiently well grounded in the older literature of their subjects, and it seems that this is one of the "problems of metabolism" which cries out for treatment and for cure. It appears to be a need in all countries.

That the new is not always the most illuminating may be learned by reading Billroth's "The Medical Sciences in the

German Universities," which, though written fifty years ago, has just been translated from the original German. The problems confronting us are the same as those of the German universities half a century ago.

A well-nigh indispensable help to research is criticism; it provokes better work. Well-trained men who settle alone in a community in which there is no searching criticism are likely to suffer a lowering of their intellectual morale. The right kind of criticism brings out appropriate response. Spoken criticism is usually not as effective as written criticism, for the latter exerts pressure from a wide audience. One will not have it said of him

> " 'Tis strange the mind, that very fiery particle,
> Should let itself be snuffed out by an article."

Your victim cries, "You have forced me to write another book"; and you may answer, "It is well."

The worst thing one may say about a laboratory worker is that his work is sloppy. It is sometimes necessary to say it. The literature is burdened with poor work. Sometimes silence is sufficient.

It should be no affront to say that theories are false, and yet a scientist is often most sensitive in the region of his theories, and the wound may be deep. One is reminded of the blind men of Hindustan who were taken to visit an elephant. One of them handled the trunk and found the elephant to be very like a rope, another felt the leg and found the elephant to resemble a tree, and so on.

"So these wise men of Hindustan disputed loud and long,
Each to his own opinion exceeding stiff and strong,
Though each was partly in the right,
Yet all were in the wrong."

I remember poking fun at the tomato as being nothing but water colored red, only to be later shown that it contained vitamins A, B, and C. All publications should contain paragraphs devoted to "the errors of the author and his critics." Criticism which is personally unkind, as was that which flourished a hundred years ago, has no place in the world today. Such criticism perhaps reached its climax in Byron's picture of the fall of Southey into Lake Avernus,

"He first sank to the bottom like himself,
And then rose to the top, like his works,
For all corrupted things are buoyed up like corks,
By their own rottenness."

We have today no Vision of Judgment which delineates the ultimate repute of our own endeavors.

In his introductory lecture to his students Carl Voit always told them, "I do not ask you to believe anything I tell you because I say it is so. I only ask you to believe those things which I can prove to you are true." And so in this lecture I will try to distinguish between known facts and the things of which dreams are made.

METABOLISM

The chemical and energy transformations in living things constitute the basic factors in metabolism work. The scope

is wide as life itself. The anatomy of the body is well known; the chemical anatomy of its constituent parts is becoming slowly known. Witness the epinephrin of Abel and Takamine, the thyroxin of Kendall. We rejoice in Levene's discovery of the chemical structure of nucleic acid. But when we contemplate the fact that blue eyes are not inherited except from blue-eyed ancestors, we are confronted with a problem transcending the scope of our analysis. The mere mention of such a problem indicates that the field to be tilled is limitless.

About a dozen years ago the expression *basal metabolism* was introduced into the literature as representing a translation of the German word *Grundumsatz* employed by Magnus-Levy. It represents the heat production of a quiet individual eighteen hours after food ingestion and in an environment which is free from thermal stimulation. Cold must not play on the skin, nor external heat raise the temperature of the body. Under these conditions the heat production manifests a marvelous constancy. During a period of eleven years the basal metabolism of E. F. Du Bois varied from the average by a maximum of ± 8 per cent and showed an average variation of only ± 3.4 per cent. The basal metabolism of a dog confined in a cage and maintained on a daily diet sufficient to supply its needs is even more constant than this. Thus Rapport[3] in our laboratory found in Dog 19 an average basal metabolism of 16.52 calories an hour in fourteen determinations during a period of fifteen months. The maximal variation was ± 3 per cent and the average variation was less than 1 per cent throughout the whole period. Since

eight alcohol checks, made in order to measure the accuracy of the respiration calorimeter during this period, showed a maximal error of ±1.9 per cent, it is evident that the slight variations in the determination of the basal metabolism of the dog may in part be due to the limitations in the accuracy of the method employed.

It is very important to realize this background of fundamental biologic behavior. A little while ago, at one of our scientific meetings, a report was made of enormous day-to-day fluctuations in the basal metabolism of dogs. When I protested against this work the representative of a well-known laboratory rose to concur with the person who had obtained the hugh gyrations shown in the level of the basal metabolism of the dogs under consideration. I could only hold my peace and wait.

It is of utmost importance to realize the constancy of the background. Resting quietly in a box at the warm temperature of 26° C. (77° F.), a dog produces from chemical energy: (1) electric currents at each contraction of the heart or of the muscles of respiration; (2) mechanical energy when the heart places blood under pressure in the arteries and when the respiratory muscles act as power on levers, the ribs, and (3) the power which maintains the vibratory movements in the various organs of the body, the sum total of which we call life. The basal metabolism measures in terms of heat production, that is, in calories, the sum of all these various physical movements of animate matter. The movements which are supported at the expense of the oxidation of fuels in the body are themselves converted into heat. That the

amount of energy necessary to maintain life is a constant is a fundamental fact of great biologic importance. I purposely reiterate this.

Against this constant background may be contrasted the results of food ingestion, of temperature influences, and of mechanical work, factors recognized since Lavoisier.

VARIABILITY OF HEAT PRODUCTION

Rubner's experiments on the influence of environmental temperature on the heat production of a dog are classical. In the presence of cold the heat production increases to meet the heat lost from the body. One cannot observe a cat fast asleep in the sun on an icy winter day without realizing the important effect which cold on the surface of the skin must have on the heat production of the animal.

Rubner[5] has recently made important contributions to this subject, which deserve to be well known. In the first place he shows that when various forms of life are exposed to a temperature of 16° C., the heat production for each kilogram of body substance is extremely variable.

METABOLISM AT USUAL ROOM TEMPERATURE

	Each kilogram at 16° C. Weight.	Calories in twenty-four hours.
A small fish	1.75 gm.	39
Yeast cells	....	73
Mouse	1.75 gm.	977
Guinea-pig	50 gm.	286
Horse	450 kg.	15

A mouse living in air at a temperature of 16° C. produces twenty-five times more heat than a fish of equal weight.

Rubner investigates the application of the surface area law in fish, amphibians, and reptiles, and finds that it does not hold.

BASAL METABOLISM OF COLD-BLOODED ANIMALS DETERMINED AT 16° C.

Fish, gm.	Calories for each square meter of surface in twenty-four hours.		Weight, gm.	Calories for each square meter of surface in twenty-four hours.
0.5	38.8	Frogs and toads	0.8–49	128
2.25	44.3	Lizard (lacerta)	110	45
3.8	26.0	Alligator	1380	47
193.0	30.4	Lizard (uromastix)	1250	29
245.0	32.5	Frogs	1.3–600	98
		Turtle	135	64
Average	33.08	Average	...	68.5

The muscular mechanism varies in different cold-blooded animals. A salmon is very active in water just above the freezing-point, whereas snakes and frogs are listless at this temperature.

RELATION OF HEAT PRODUCTION TO SURFACE AREA

The picture of the relation of the heat production to the surface area changes completely when one considers the relations existing between various mammals. Rubner's figures are as follows:

	Calories for each square meter of surface in twenty-four hours.
Man	1042
Pig	1078
Dog	1039
Rabbit	917
Guinea-pig	1131
Mouse	1181

These comparative figures are valid, Rubner remarks, despite the criticism which some individuals feel themselves forced to make. Why is it that the development of a species is so ordered that its requirement of energy is mathematically proportional to the surface area? If surface area is not related to the quantity of energy produced, what is it that brings about these definitely ordered relationships?

Considering the development of offspring, Rubner points out that the aim of growth is to produce a new organism akin to the parent, not only in form and size, but also similar in energy production, in consequence of which a gradual reduction in the heat produced for each kilogram must accompany growth. When the child reaches the same size and shape as the parent, it will also have the same energy production for each square meter of surface, even though the surface area has no constant influence on metabolism. The surface area may be a valid method of measuring metabolism even though it may not be the direct cause of the heat production. Also the cell mass of animals of different sizes manifests an adaptation to different surface areas. The process of manufacturing a warm-blooded animal from a cold-blooded one is thus pictured by Rubner:

1. There would be a development of feathers or hair and, in mammals living in cold water, of subcutaneous fat, all of which are bad conductors of heat. Further, the *physical regulation* of body temperature would be established, by which the distribution of blood at the surface of the skin is regulated in order to control the loss of heat. The surface area here becomes a factor in the behavior of the animal.

In hot weather increased evaporation of water from the lungs and skin would cool the body, a mechanism possessed even by frogs and turtles. Through the evaporation of water a frog exposed to a temperature of 30° C. maintains a body temperature of 20° C. An animal provided with the foregoing mechanical arrangements may maintain the normal body temperature of a warm-blooded animal if the surrounding environment has a sufficiently high temperature.

2. To complete a warm-blooded animal, however, requires the creation of a neuroregulatory center in the brain in control of the mechanism of *chemical regulation* by which heat production is increased in the presence of cold. Without this mechanism a warm-blooded animal would be a very incomplete creature whose existence would be strictly limited to life at a high environmental temperature, and when exposed to cold would not be able to maintain its heat production except through muscular movements.

We recall the cat basking in sunshine in zero weather and contrast its life with that of the alligator, which would freeze under like conditions. Rubner compares the metabolism of a marmot weighing 3.15 kg. during its winter sleep when its body temperature was 10° C., with that of the same animal awake with a body temperature of 36.7° C.

METABOLISM OF A HEDGEHOG

	Body temperature.	Calories for each kilogram.	Calories for each square meter of surface.
Asleep.....	10° C.	2.87	47.5
Awake.....	36.7° C.	67.74	1160.0

At a body temperature of 10° C. the marmot had a heat production for each square meter of surface comparable to

that of an amphibian or a reptile, but when its body was warmed to the normal mammalian temperature the heat production rose twenty-fivefold and conformed to the usual surface area standards, as given by Rubner. The figures recall the comparison between the fish and the mouse of equal weight.

I have spent some time on this subject because the surface area standard as a measure of the basal metabolism has been met with flat denial in certain distinguished quarters without, it seems to me, giving due weight to all the facts of the case. I would have you bear always in mind the constancy of the basal metabolism and its general conformity to the law of surface area.

EFFECT OF PROTEINS AND PROTEIN DERIVATIVES ON HEAT PRODUCTION

The ingestion of protein always, and of fat and carbohydrate when given in quantity, increases the heat production. For example, I would call your attention to the following results taken from the work of Weiss and Rapport[7] when Dog 19 received the same diet factors on different occasions:

Date.	Calories.	Increase over basal of 16.48 calories, per cent.	Food, gm.
March 21, 1923	19.98	21.2	Glycine, 10
December 10, 1923	19.99	21.4	Glycine, 10
March 7, 1924	20.05	21.7	Glycine, 10
February 9, 1923	21.19	28.6	Gelatin (6 gm. N)
April 30, 1923	21.41	29.9	Gelatin (6 gm. N)
December 7, 1923	21.62	31.2	Gelatin (6 gm. N)
March 18, 1924	21.01	27.5	Gelatin (6 gm. N)
	21.31	29.3	

This dog, with a body weight of 9 kg., when given 10 gm. of glycine or 0.1 per cent of its weight, reacted on three different occasions, two of which were a year apart, so that the heat production rose by 3.50, 3.51, and 3.57 calories. The average is 3.527 calories, with a maximal variation of slightly over 1 per cent. After giving 38.7 gm. of gelatin to the same dog on four different occasions the average increase in the heat production was 4.80 calories, with a variation of ± 6.6 per cent. These experiments were made at different intervals during a period of thirteen months.

In two other experiments 43.7 gm. of casein reacted to increase the dog's heat production by 5.06 calories within a $\pm$ variation of 2 per cent. Similar results after giving glucose are on record.

Thrown against the background of a constant basal metabolism, we see outlined a definite quantitative reaction measured in terms of extra heat production whenever protein or such a product of protein metabolism as glycine acts as a stimulus on the cells of the organism. The heat produced by mixing a given quantity of water and sulphuric acid together in a test-tube is scarcely more exactly measurable than are these reactions of living cells to the amino-acids or polypeptids which reach them after meat or kindred substances are taken as food.

In the light of such established facts one may investigate the doctrine of the specific dynamic action of protein. Rubner first showed that if the quantity of ingested protein was increased the heat production was increased in proportion. I have shown the same of glycine. But when glycine, of

which 10 gm. alone raised the heat production of Dog 19 20 per cent, is mixed with 40 gm. of gelatin or with the same amount of casein, either of which alone raised the heat production 30 per cent, the total increase in heat production remained exactly the same as though no glycine had been added, that is, 30 per cent.

Here, then, is a real problem of metabolism. We remember the older work of Folin, how ingested glycine passes through the liver and may be recovered in the muscles; also the work of Van Slyke and of Fisher and Wishart, which showed that when meat itself is given amino-acids do not accumulate in the liver or muscles. This suggests that they are re-formed into protein in these localities. And now comes the recently published work of London[1] which shows that after a meal of meat the blood of the hepatic vein is much richer in polypeptid nitrogen than is that of the portal vein. So we may ask ourselves, does glycine form with the broken products of casein a polypeptid of such a nature that it exerts no stimulating action? Or, as Professor Rubner asks after commenting on this matter in a private letter to me, is polypeptid nitrogen, when present in the blood, used by the cells to the exclusion of such an artificial product as glycine? So here is a problem in metabolism, something for you to explain if I do not find out the reason first. We have known about it for two years, but have found no explanation.

METABOLISM OF FAT AND CARBOHYDRATE

Another problem[4] of metabolism which has recently interested us greatly is that of fat production in the hog. Wier-

zuchowski and Ling have recently repeatedly found respiratory quotients above 1.5 in a young hog fed with starch and sugar. Even the day following the food ingestion the quotient has been found to be 1.4. This means that a hog of 10 kg. may readily form 100 gm. of fat daily from 270 gm. of starch. A daily increase of body fat of 1 per cent of the total body weight shows what an important fat factory the hog really is. When carbohydrate is converted into fat there is a large elimination of carbon dioxid without a corresponding demand for oxygen or a corresponding increase in heat production. Hence, the proportion of the volume of carbon dioxid eliminated to that of oxygen absorbed becomes greater and greater the more fat there is produced. Since the fat production of the hog is proportional to its respiratory quotient, we may patiently await the dawn of that scientific era when the price at which young pigs are sold is proportional to their respiratory quotients! Seriously speaking, however, this is a problem for extensive study on the part of some one of our many agricultural experiment stations. An evident corollary of the discovery that during the fattening period the respiratory quotient may rise to 1.5 is that a calculation of the heat production from the carbon dioxid elimination under these conditions may lead to very gross errors.

The method by which 270 gm. of cornstarch may be converted into 100 gm. of hog fat is unknown, but it is a problem of greatest interest. It was from Hofmeister's laboratory that the suggestion came that carbohydrate lost carbon dioxid and produced acetaldehyde and that these molecules

of acetaldehyde condensed, forming fatty acids of higher and higher order. Later Neuberg proved that acetaldehyde molecules were intermediary products in the alcoholic fermentation of sugar. And more recently Neuberg has presented evidence that this highly reactive substance, acetaldehyde, is also a product of mammalian metabolism. A. I. Ringer was the first to associate acetaldehyde with the mechanism of antiketosis, but Philip Shaffer finds that glycolaldehyde and not acetaldehyde is the effective metabolite concerned in the phenomenon. The interplay among the broken molecules of starch from which fat is formed takes place at the cost of little energy. There is almost no waste of value here. After the living expenses of the pig itself have been paid for, almost all the energy of the starch which is taken in excess of these living expenses is recovered in the form of manufactured fat, created by a process which will some day become clear. We wish to find out all about this process of manufacture and have it as clear and as evident as the manufacture of Ford cars.

One of the manifold byways of practical application through scientific understanding is shown in the administration of glucose in cases of nervousness and prostration, as reported by Parker and Finley.[2] From hearing of the symptoms of insulin hypoglycemia Mrs. Parker concluded that the nervous irritability and excitement manifested by some school children might be due to hypoglycemia. The reported results show that when two or three teaspoonfuls of glucose (commercial exose) are given in water before rising and also between meals in lemonade, sometimes as often as

six times a day, the nervous symptoms disappear and school work is accomplished without strain. Between 40 to 100 gm. were given daily. In women who feel nervously fatigued the same course of treatment may bring about the sensation of vigorous well-being. During the war, when many of us lived on a restricted diet, lack of physical vigor may often have been due to hypoglycemia. Perhaps the pancakes and New Orleans molasses of our ancestral breakfast tables had their scientific justification. In England tea (containing sugar) is often served in the early morning before arising, and afternoon tea results again in raising the blood-sugar level.

A certain level of blood sugar appears to be necessary for the proper functioning of the muscle. When the muscle contracts the reaction, glycogen ⟶ glucose ⟶ lactic acid, takes place, to be followed in the recovery phase by the reaction, lactic acid ⟶ glucose ⟶ glycogen, as clearly expounded by Hill and by Meyerhof. Doctors Deuel and Chambers, in our laboratory, have discovered that a dog weakened by the combination of long fasting and diabetes may be greatly restored in strength a few minutes after giving a few grams of glucose. Correction of the hypoglycemia restores the muscular power even though none of the glucose can be oxidized by the diabetic organism.

From this little story about glucose we can see the inescapable relation between the theoretic and the practical. Science ever points the way; commercial prospectors gain the financial reward.

In this discourse I have recited a few facts which seem to

me to be of interest and importance. It is fortunate that others may think differently or may entirely disapprove, for along such lines come discussion, experiment, and advance in knowledge. As "der alte Voit" used to say, "It matters not *who* is right, provided the *truth* becomes known."

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THE PROPORTIONS IN WHICH PROTEIN, FAT, AND CARBOHYDRATE ARE METABOLIZED IN DISEASE

EUGENE F. DU BOIS

During the last twelve years there has been a rapid growth in the study of the respiratory metabolism. This has been made possible largely through the various types of apparatus developed by F. G. Benedict. Clinicians have been interested chiefly in the basal metabolism because it is an aid in diagnosis and treatment. They have neglected the valuable information furnished regarding the proportions in which the different foodstuffs are oxidized in health and disease. It is only through a careful study of these proportions that we can estimate the various processes which are taking place within our patients' bodies. We pay a great deal of careless attention to the diets which we expect the patients to utilize, but, as Richardson[22] points out, we seldom have any idea of what really happens after the food is swallowed. Perhaps I should have selected the following title for this paper: "The food that the patient metabolizes is seldom the same as that which the doctor orders in the diet."

The human body contains large storehouses of protein and fat. Rubner estimates that a man weighing 73 kg. contains 13.5 kg. of protein with its 2200 gm. of nitrogen. The fat,

of course, varies greatly according to the state of nutrition, but the average man contains many pounds of fat. The carbohydrate stores are much smaller. To the best of our knowledge the average man stores in his liver and muscles 250 to 400 gm. of glycogen. We may liken the body to a yacht with three food lockers: two large ones for protein and fat, and one very small one for carbohydrate. Since the crew consumes carbohydrate by preference it is obvious that the small locker will be emptied quickly unless the stores are replenished at frequent intervals. If they are not replenished the crew must get along with the protein and fat, and fortunately the stores of these are large enough to last them many days. These same protein and fat lockers will be drawn on if the steward of the yacht does not bring on board sufficient quantities of fresh food. You can see that the crew may subsist on a ration quite different from that which the steward purchases from day to day.

In the case of the human body if we really want to know what is taking place we must review certain well-known laws of nutrition and, above all, adopt a somewhat different viewpoint. Perhaps the best method of doing this would be to start a prolonged metabolism experiment on a normal man. We shall assume that he is the ideal experimental subject, the man who can take any sort of diet without complaining. During our experiment we can determine his protein metabolism by collecting the urine and finding its nitrogen content. We know that each gram of urinary nitrogen in a given period means that 6.25 gm. of protein have been metabolized in about the same period. We can measure his

respiratory metabolism and find his basal rate and his total calories and, what is even more important for our present study, we can determine his respiratory quotient. I shall not discuss the details of the calculation, but merely state that if we divide the liters of CO_2 excreted by the liters of O_2 consumed we can estimate the grams of fat and carbohydrate metabolized. In this manner it is possible to determine the proportions in which he actually oxidizes protein, fat, and carbohydrate. Under ordinary circumstances our reckonings are sufficiently exact for practical purposes. We must remember that there is an error of 1 or 2 per cent in all metabolism calculations on account of slight differences in the figures used for atomic weights, factors for changing weights of gases to volumes, differences in caloric values, and so forth. There is also a certain lag in the excretion of nitrogen after the protein has been metabolized. The respiratory quotient is not reliable under changing conditions of lung ventilation, in acidosis, and in a few other conditions. I shall not discuss these possible errors at the present time, but shall leave them for a chapter in the mythical "Encyclopædia of Artifacts." This important compendium, if anyone attempts its publication, will fill many volumes, and the largest will be devoted to metabolism. I shall try to avoid the artifacts and base my discussion chiefly on the results of experiments made with the respiration calorimeter and other forms of apparatus whose reliability under the given conditions is well understood.

In our laboratory our faith in the respiratory quotient is based not only on the background of theory and authority

but also on many forms of evidence which have been obtained in work with the respiration calorimeter. When alcohol is burned the quotient obtained corresponds closely with the theoretic quotient. In phlorizin diabetes and complete human diabetes theoretic quotients are obtained with sur-

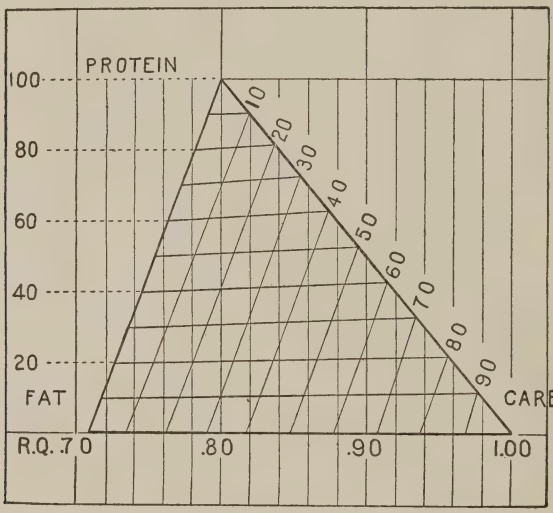

Fig. 9.—Diagram showing the percentages of calories derived from protein, fat, and carbohydrate according to the respiratory quotient. The base line gives the total respiratory quotient; the ordinates reading on the left-hand side give the percentage of calories from protein; the diagonals reading on the right of the triangle give the percentage from carbohydrate.

prising accuracy. In normal subjects and in patients with many diseases the results for the calories produced, as calculated from the respiratory quotient, correspond within 1 or 2 per cent with the actual findings of direct calorimetry. Lusk's work on the intermediary metabolism in experiments

on dogs is based largely on the respiratory quotient, and the agreement with the method of direct calorimetry would be hard to explain if there were gross errors in the interpretation of quotients. Perhaps one of the strongest arguments is the recent work of Richardson and Ladd[23] who have checked the respiratory quotient against the threshold of ketosis. We must remember that this work was performed in hourly periods in a respiration chamber, but there is every evidence that even short periods with the technic used by Benedict, Boothby, Krogh, and their associates is exceedingly accurate.

It has always been rather difficult to visualize the respiratory quotient and show its significance. Fortunately the medical profession is now so familiar with graphic methods that we may resort to the use of diagrams. In Figure 9 a triangle has been constructed somewhat after the manner of the food triangles of Fisher.[13] The base has been drawn to represent the respiratory quotient and the corners are assigned to fat, protein, and carbohydrate.[11] Fat has a quotient of 0.707, protein 0.801, and carbohydrate 1.00. Each corner represents the theoretic point in which all of the calories are derived from one of these three substances. Of course this never happens in real life, for we derive our calories from the oxidation of mixtures. The horizontal and diagonal lines represent the percentages derived from each. The spacing of these lines is almost but not quite equal since the calculation involved is rather complicated. The interpretation also seems complicated at first sight, but will become easier with the subsequent diagrams.

In Figure 10 is shown the position of the basal metabolism of the normal control E. F. D. B. who happens to be the subject observed most often in the calorimeter. It will be noted that when he omits a breakfast and is studied at perfect rest between 11 a.m. and 1 p.m. he derives about 19 per cent of his calories from protein, 31 per cent from carbohydrate, and the remaining 50 per cent from fat. Of course

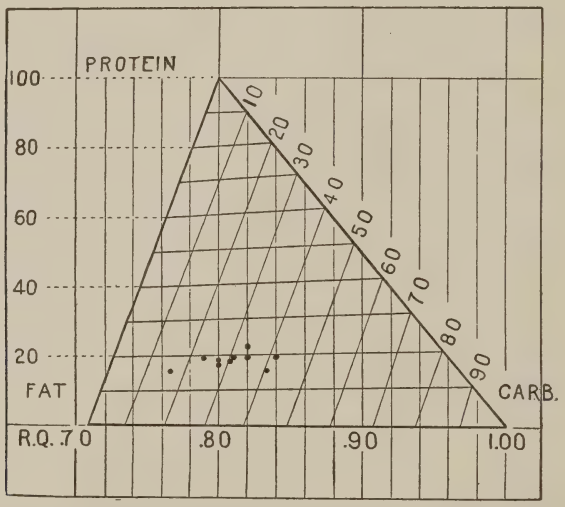

Fig. 10.—Dots showing the position of the basal metabolism of one normal man as determined in ten calorimeter experiments.

the exact proportions depend to a large extent on his food of the previous day. In Figure 11 (D) are shown the effects of taking 200 gm. of glucose for breakfast at 10 a.m. The first calorimeter period began one hour later. At this time the respiratory quotient was 0.95, showing that he was deriving 80 per cent of his calories from carbohydrate. The

urinary nitrogen proved that he was obtaining the other 20 per cent from protein. For the next two hours the percentages were very slightly changed, but in the fourth hour the quotient rose to 1.00, indicating that some of the carbohydrate was being transformed into fat.

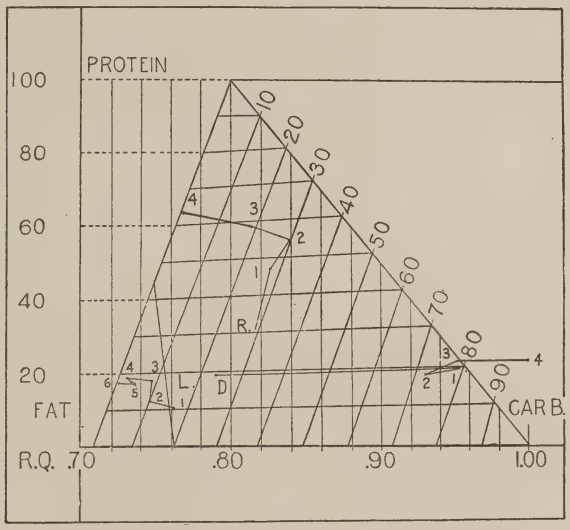

Fig. 11.—Triangle used to show changes in metabolism: R, the effect of a large protein meal; D, the effect of 200 gm. of glucose; L, the first six days of starvation.

On another day a moderate sized protein meal containing 10.5 gm. of nitrogen caused the protein metabolism to increase until in the fifth hour it furnished 29 per cent of the calories, carbohydrate contributing 25 per cent, and fat the remaining 46 per cent. This test is not illustrated in Figure 11. Much more striking results were obtained in the case of an achondroplastic dwarf[1] selected because his arms and

legs were very small and his appetite and stomach very large. This little man who weighed but 90 pounds ate chopped meat steadily for one hour and consumed a total of 662 gm. of beef with its 23.2 gm. of nitrogen. In other words, he took for breakfast about twice as much protein as the ordinary man eats in one day. The calorimeter period which began one hour after this unusual meal is shown in Figure 11 (R). At

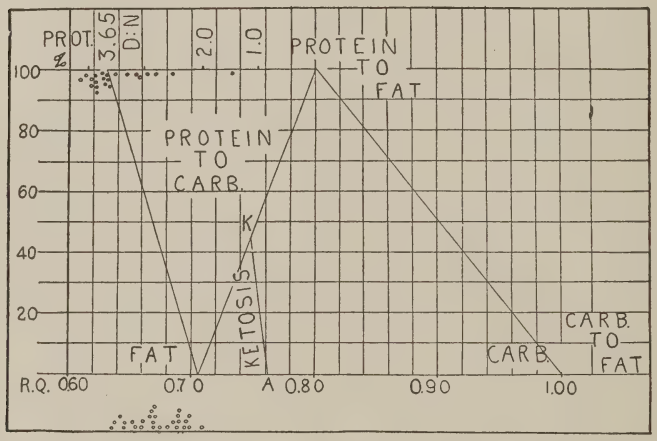

Fig. 12.—Zones of metabolism. The dots under the R. Q. line show the lowest respiratory quotients obtained in severe diabetes. The dots under the D : N line show the highest D : N ratios found in the literature.

this time he was obtaining 47 per cent of his calories from protein. Between the fourth and fifth hours he was deriving 64 per cent from this source and the quotient of 0.77 indicated that fat was supplying 34 per cent.

In Figure 12 there have been added certain zones outside of the triangle. When carbohydrate which contains a large proportion of oxygen is transformed into fat with little

oxygen the respiratory quotient rises above unity, sometimes to 1.2 or even 1.5. The zone of this transformation lies, therefore, to the right of the triangle. To the left of the triangle is found another zone which is never invaded by normal men. We know that 100 gm. of protein can furnish in the metabolism of the living body about 58 gm. of glucose. In severe diabetes this glucose is excreted in the urine and the respiratory quotient falls below 0.707. Since protein rarely furnishes more than 30 to 40 per cent of the total metabolism we seldom approach the upper portions of this zone or reach the lowest theoretic quotient, 0.63.

There are probably a number of chemists in this audience who are looking for the zone in which fat is transformed into carbohydrate. If this really took place the area would lie to the left of the main triangle and would extend far to the left. We would obtain many respiratory quotients below 0.65, especially in cases of severe diabetes. It is quite easy for the chemist to see how glycerol would be changed into glucose and there is much evidence to prove that this actually occurs, but glycerol forms such a small part of the fat molecule and the quotient is so little affected that the process cannot be detected in the quotient. The chemist can also write reactions showing the theoretic transformation of fatty acid into glucose, and most investigators in Europe believe that this actually occurs. Personally, I am quite convinced by the arguments of Lusk and other American biochemists that this has never been proved. If it did occur, all the modern theories of antiketogenesis would fall by the board. If fat were changed into glucose, this glucose

would be excreted in cases of complete diabetes, giving us many respiratory quotients below 0.65 and many D : N ratios above 3.65. In Figure 12 I have plotted the most reliable data which could be found in the literature. You will note that few are beyond the limits mentioned and these slight discrepancies could easily be due to experimental error. This means that the complete diabetic excretes no sugar that does not come from carbohydrate or the carbohydrate portion of the protein molecule or from glycerol. It also means that he does not transform an oxygen-poor substance like fatty acid into an oxygen-rich substance like carbohydrate. We can only use the condition of complete diabetes to make these deductions, because all normal men have carbohydrate available for oxidation and we cannot prove that it was not derived from fat. It is quite possible that fat passes through some carbohydrate-like stage as it is oxidized. All we can say is that it passes through this stage so quickly that it makes no difference to the practical results. We may illustrate this point by returning to the yacht with its three food lockers. If the crew called for carbohydrate and the carbohydrate locker were empty the steward might take some fat and place it for an instant in the carbohydrate locker before serving it. He might call it carbohydrate because it had been in that locker, but for all practical purposes it would remain fat.

Having thus explored the territory which surrounds the triangle it is necessary to return to the affairs of every-day life and study the factors which affect the position of the metabolism within the triangle. In a previous lecture of

this series Hill[14, 15] has shown, by means of his brilliant experiments, that in periods of short violent exercise the energy is derived solely through the oxidation of carbohydrate. He has emphasized the fact that respiratory quotients are falsely high during exercise and falsely low during recovery so that we must average the CO_2 and O_2 of a long series of consecutive periods which cover both phases. He has shown that we can subtract from such a work period the basal CO_2 and O_2 and find the quotient of the work itself, together with its recovery phase. Protein seems to play but little part as a source of energy for muscular work. This was shown long ago by Voit and confirmed in the classical experiment of Fick and Wislicenus,[12] who found no increase in the nitrogen excretion when they climbed a high mountain in Switzerland. Kocher[16] has shown the same thing even more clearly by placing himself on a nitrogen minimal excretion of 2.9 gm. a day, obtaining a negligible increase on the day when he walked 60 kilometers. Thomas[29] has obtained similar results. On the other hand, it is quite evident that protein can furnish a considerable portion of the energy for muscular work in carniverous animals. It is also evident that fat must furnish the chief source of muscular energy in the case of individuals like the Eskimos who consume practically no carbohydrate yet are capable of great feats of exertion. It is estimated[19] that some of the Eskimos derive only 8 per cent of their calories from carbohydrate since they consume only 50 or 60 gm. a day. This is obviously too small an amount to carry them on long hunting expeditions. In respiration experiments performed on individuals who are

taking moderate or long-continued exercise there is surprisingly little change in the quotient when we discount the sudden driving off of carbon dioxid due to the lactic acid formation and suddenly increased pulmonary ventilation.

In New York our interest in this phase of the subject was greatly stimulated by A. V. Hill's visit to this country, and we immediately recalculated all of our calorimeter experiments in which the subjects had performed mild amounts of exercise such as might readily be accomplished by hospital patients. We also had at our command a certain number of observations made by Barr, Cecil, and Du Bois,[2] in which the patients had shivered quite violently during chills caused by malarial paroxysms or by the intravenous injection of foreign protein. In all of these experiments the subjects were observed for more than an hour after the muscular work so that we could obtain the phase of recovery during which the excess of lactic acid was reconverted into glycogen. It was, therefore, possible to find the respiratory quotient of the work increment. Doctors Richardson and Levene have kindly allowed me to add calculations taken from their article on exercise in diabetes which is as yet unpublished. It will be noted that there is little difference between the basal quotients and those of the exercise increment, the average for the former being 0.835, for the latter 0.782. This means that these patients during mild exercise consumed fat and carbohydrate in about the same proportions as when they were resting.

At the beginning of this lecture I emphasized the fact that the diet on any given day might differ greatly from the

TABLE 1

EFFECT OF MILD EXERCISE ON R. Q.

Subject		Exercise		Basal R.Q.		Exercise Increment
E.F.D.B.	Normal	Shivering	34 min.	0.83		0.86
E.F.D.B.	"	"	35 "	0.81		0.87
Chas. R.	Congenital absence of sweat glands	"	34 "	0.88		0.63
George S.	Malarial chill	"	34 "	0.78		0.83
Joseph McC.	Typhoid vac.	"	30 "	0.83		0.71
" "	" " "	"	32 "	0.89		0.79
			Average	0.835	Average	0.783
David L.	Diabetic Mild exercise		40 min.	0.80		0.81
Morris G.	" "	"	59 "	0.77		0.74
Ray H.	" "	"	71 "	0.85		0.81
Jervis B.	" "	"	97 " ?	0.83		0.72
" "	" "	"	63 "	0.80		0.73
Nicholas S.	" "	"	26 "	0.80		0.73
James D.	" "	"	56 "	0.80		0.77
Gerald S.	" "	"	10 "	0.76		0.84
		Average diabetic		0.80		0.768

food actually metabolized. Conditions, however, are entirely different in normal individuals subsisting for months and years on any fairly uniform dietary which is sufficient to supply the bodily needs and prevent loss of weight. In the course of a year the average metabolism of the race must correspond quite closely with the average composition of the diet. We can, therefore, study with profit the standard dietaries of different races, as shown in Figure 13. It is interesting to note that the Eskimo takes five times the amount of protein eaten by the Bengali. We must, of course, remember that the position of the metabolism on such a

diagram changes after each meal and that the points shown are merely averages.

On this chart there are certain zones which are not ordinarily reached in daily life. Of particular interest is the zone of low protein metabolism such as can be obtained in experiments on the nitrogen minimum. Most Americans

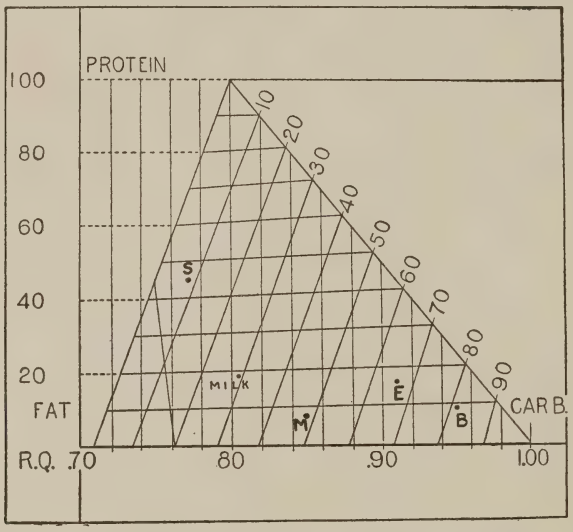

Fig. 13.—Position of various racial dietaries: E, Voit's European "Standard." B, Bengali. M, Maine lumberman. S, Eskimo.

under ordinary conditions derive about 15 to 20 per cent of their calories from protein, some as little as 10 per cent. Since the protein metabolism does not increase with muscular exercise, it is obvious that a man who runs a race, increasing his total metabolism ten times or more, will, during the period of muscular exertion, derive only 1 or 2 per cent of his calories from protein. A great deal of informa-

tion has been furnished by observations on the protein minimum. Klemperer, Sivén, Landergren, and others gave men an ample diet, rich in carbohydrate, containing 2 to 2.5 gm. food nitrogen. On this diet the men excreted 3 to 4 gm. of nitrogen daily. Thomas obtained even lower results and actually placed himself on nitrogen balance at the very low level of 2.2 gm. of nitrogen a day. Thomas' experiments

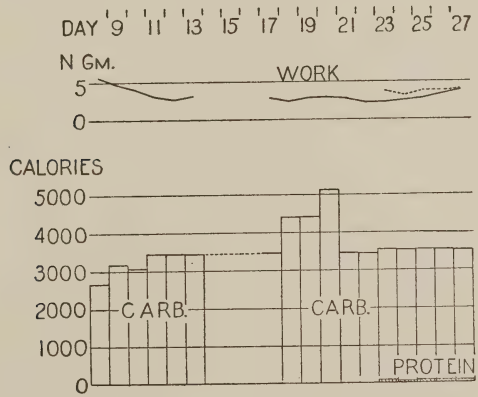

Fig. 14.—Experiment of Thomas on the nitrogen minimum. The continued line shows the urinary nitrogen. The dotted line represents food nitrogen.

are illustrated in Figure 14. His work demonstrates the fact that carbohydrates are more efficient than fats as sparers of protein. It also proves that we can give small amounts of protein in a diet without increasing the protein metabolism. Altogether, his long experiment is the best demonstration of the "wear and tear quota" of Rubner, that minimal breaking down of protein which seems to be necessary for life.

Having considered the effects of foods we must next take

up the factor of undernutrition since it enters into the picture of almost all patients who are seriously ill. The most complete experiment that has ever been made in starvation was performed by Benedict[4] and his associates. They had the unusual opportunity of studying the subject Levanzin

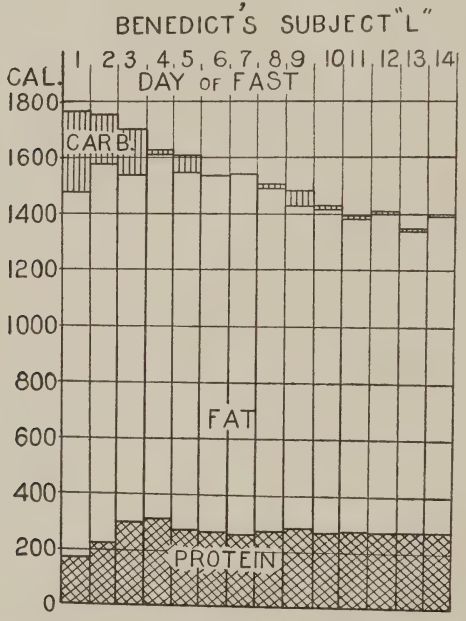

Fig. 15.—Benedict's fasting subject, Levanzin. Materials oxidized during first fourteen days of starvation.

under almost ideal conditions since he slept in the respiration calorimeter every night during his thirty-one-day fast. Additional respiration experiments were made in the daytime. The results of the first fourteen days are shown in Figure 15. It will be noted first that there was a gradual fall

in the total heat production. The amount of carbohydrate oxidized was quite considerable on the first day but decreased rapidly as the glycogen stores were depleted, until after the fifth day only traces were consumed.

The protein metabolism was comparatively low in the first day of the fast and he only excreted 7.1 gm. of nitrogen in the urine. When the carbohydrate decreased the protective effect of this substance on the protein destruction was diminished to such an extent that the nitrogen elimination rose to 11.9 gm. Later it fell to the level of about 8 gm. a day, partly on account of the diminished total metabolism, partly perhaps because the organism was adapting itself to the new conditions. It will be noted in the chart that the chief source of heat was the fat derived from his rather ample stores, although Levanzin was by no means obese at the beginning of his fast.

In this particular subject Benedict had the unusual opportunity of studying the amount of glycogen which had been stored in the liver and muscles at the beginning of the fast. The muscles contain about 0.3 per cent of glycogen, but the liver is the chief storehouse since its glycogen content may be very high. A dog's liver may hold 18.7 per cent of its weight as glycogen and there is no reason to suppose that this level cannot be reached in man. The calculation of the total carbohydrate storage in man is made by noting the percentage of calories furnished by carbohydrate during the respiration experiments and assuming that this percentage holds good for the periods when the subject is outside the calorimeter. In Levanzin's case Benedict found that 201

gm. of glycogen were available. It is doubtful if we shall ever know how completely the glycogen stores are exhausted in the starving human subject. Experiments on phlorizinized dogs by Lusk and others have shown that it is necessary to use not only prolonged fasting but also adrenalin and periods of shivering or other violent exercise which mobilize the glycogen so that it is available for excretion by the kidneys. Richardson and Ladd,[23] in their observations on human diabetes, have obtained indications that there may still be considerable amounts of glycogen stored in the body, even after periods when there has been little or no evidence of carbohydrate metabolism.

Complete starvation is not frequently encountered in the clinic, but partial undernutrition is almost universal in patients who are seriously ill. Normal men on inadequate diets show a gradual fall in the basal metabolism and, as a rule, a rather low nitrogen output. The materials that they actually consume in their bodies depend largely on the food that is administered. If they are given protein alone, the nitrogen excretion will rise to a point somewhat above the nitrogen intake and they will never attain nitrogen equilibrium. This was shown in the experiments of Thomas.[29] If they are given fat alone, there will be a sparing of body fat but a depletion of the stores of carbohydrate and protein. If they are given carbohydrate alone, the fat stores will be protected and if the caloric intake is equal to the total heat production, there may be an almost complete protection of the fat. Under such conditions the protein metabolism will be greatly diminished and the nitrogen minimum attained,

as in the experiments of Thomas. If carbohydrate alone is given, but in amounts which do not cover the total requirement, the sparing of fat and protein will, of course, be much less marked. As a rule, undernourished subjects are given mixtures of food, and the level of the carbohydrate and protein metabolism will depend on the amounts of these substances ingested. In all cases of undernutrition fat must furnish the rest of the calories and this fat is obtained partly from the food but chiefly from fat which has been previously stored in the body.

Let us return once more to the simile of the yacht with its three food lockers. If this yacht sails on a long voyage without touching port, the carbohydrate locker will be almost emptied in the first few days. The protein locker will be drawn on moderately each day and the bulk of the food will come from the fat stores. If the yacht does touch port at more or less regular intervals and the steward is not able to secure sufficient and proper rations, the crew will get along as best it can, utilizing completely the fresh supplies, whatever they might be, but drawing when necessary upon the stores which filled the lockers at the beginning of the voyage.

We must next consider the proportions in which fat and carbohydrate are oxidized and their relationship to ketosis. The subject has been made so familiar to you by the recent brilliant work of Woodyatt[31, 32] and Shaffer[27] that I shall not try to discuss the chemical aspects. Rosenfeld, in 1885, said, "The fats burn in the fire of carbohydrate." It has long been known that the complete oxidation of fat in the body could

not be obtained unless some carbohydrate were being oxidized at the same time. If fat is not completely oxidized, aceto-acetic and beta-hydroxybutyric acids are left in the tissues, causing the condition known clinically as ketosis. Carbohydrates are called antiketogenic because in their oxidation they prevent the formation of the ketones or aid in their complete combustion. Woodyatt,[31] in 1910, suggested that 1 molecule of aceto-acetic acid reacted with 1 molecule of an alcohol or glucose. The experimental work and calculations of Shaffer and of Woodyatt[32] have confirmed this relationship, although there is some evidence[17, 18, 30] that 1 molecule of glucose may be able to take care of more than 1 molecule of fatty acid. When the available carbohydrate is insufficient the organism "smokes" like a gasoline engine with an improper mixture.

It is possible to indicate this zone of ketosis on the triangle, and Figure 12 shows the line where ketosis appears as the carbohydrate metabolism is diminished. This line corresponds to the equimolecular ratio of Shaffer and Woodyatt's fatty acid-glucose ratio of 1.5. At the right hand border of this zone the ketosis is mild. Near the extreme fat corner it is severe. The starving man Levanzin was within this zone after the first day of his fast. The highest degrees of ketosis are, of course, found in diabetes.

It seems possible at the present time to ascribe all the various phenomena of diabetes to a diminution in the secretion of insulin by the pancreas. In mild cases, in which the loss of function is slight, the metabolism is not very different from that of normal men. In cases of moderately

severe diabetes the fat metabolism must predominate and only a small percentage of the calories is derived from carbohydrate. In cases of severe diabetes the protein metabolism is affected, since the diabetic organism can no longer utilize the carbohydrate portion of the protein molecule. The patient with so-called "complete diabetes" excretes 58 gm. of glucose for each 100 gm. of protein metabolized. He can oxidize practically no glucose from any source to aid in the metabolism of the fatty acids. As a result the formation of aceto-acetic and betahydroxybutyric acids is enormous.

The symptoms of severe ketosis are well known: deep respiration, weakness, nausea, somnolence, coma. The symptoms of mild ketosis are much less marked, but diabetics and normal men on a low carbohydrate diet show weakness, lassitude, inability to perform much mental or physical labor, and lack of resistance to infection. I am inclined to believe that there may be a distinctly lowered resistance to the effects of disease in the zone where the organism just escapes a ketosis, but I do not know of any exact data on this point.

The diabetic organism exists chiefly on fat. If the patient is starved he lives on body fat. His fat metabolism is not necessarily increased by giving him fat in his food. This was shown by Richardson and Mason[24] (Fig. 16). The fat metabolism can, however, be reduced by means of fasting which reduces the total metabolism. If the carbohydrate tolerance remains fixed this improves the fatty acid-glucose ratio. Nowadays it is possible to increase the carbohydrate

tolerance by means of insulin and this gives us even a better means of improving the ratio and avoiding ketosis.

Obese patients are often placed on very low diets in order to get rid of the surplus fat. In such cases it is necessary to

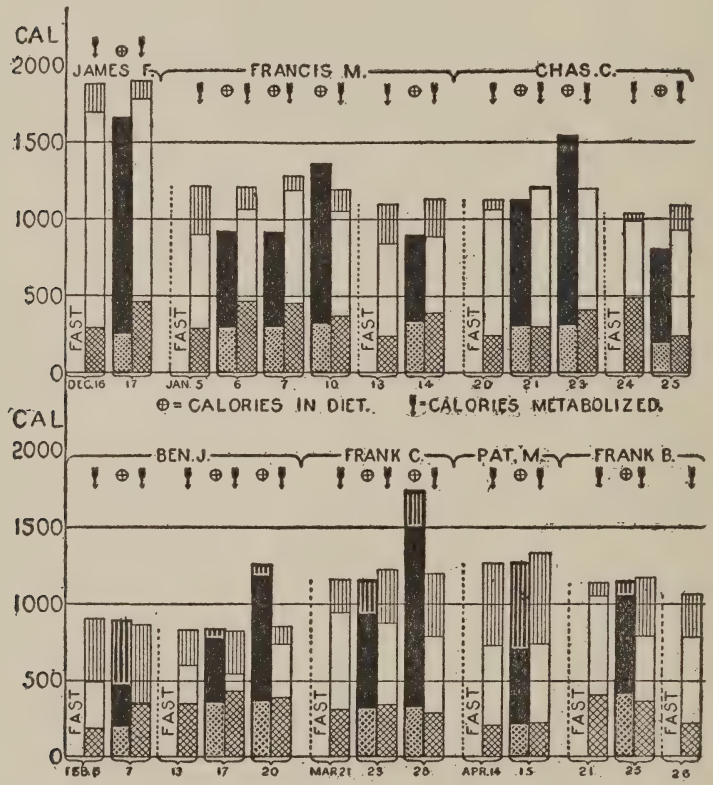

Fig. 16.—Observations of Richardson and Mason on the effect of food in diabetes. The columns under the circles represent the food given in the diet. The columns under the arrows represent the materials actually metabolized. The cross-hatched areas are the protein calories, the vertical lines represent the carbohydrate calories. Fat fed is shown in solid black, fat metabolized, in solid white.

estimate the total metabolism and see that enough carbohydrate calories are supplied to balance the large metabolism of fat.

Our diets are voluntarily restricted in obesity, but are restricted by stern necessity in almost all patients who are seriously ill. Loss of appetite, repugnance for food, nausea, and vomiting force the doctor to resort to low diets. Sometimes he is quite content to give the stomach a rest for a few days. This, however, does not give the metabolism a rest. The metabolic picture is just the same as in the starving man, Levanzin. There is a slight fall in total metabolism, and a rapid exhaustion of the glycogen stored in muscles and liver. The organism passes into the zone of ketosis and the evil effects of starvation are added to those of the disease.

Fortunately, it is almost always possible for skilful nurses to give some food. The most important food is carbohydrate because this will replenish the glycogen stores and tend to diminish destruction of body protein. Protein food is of next importance. Fat comes last of all because the human body has large enough stores to last for many days, and if these are depleted during illness they can be refilled during convalescence.

Experienced physicians have made a consistent effort in recent years to administer carbohydrate by mouth or glucose by vein or rectum. Even small amounts of glucose may be of great service. In diabetes we struggle hard to secure the metabolism of each additional 10 gm. of carbohydrate. When the patient who has been on a diet of 20 gm. increases

his tolerance so that he can take 30 gm. there is great rejoicing on the part of the physician. In a medical or surgical patient on desperately low rations we should likewise rejoice over each additional 10 gm. that is retained within the body. If it is once absorbed into the blood it will be metabolized.

As I have said before, the factor of undernutrition plays an important part in the metabolic picture of every patient who is seriously ill. There are, of course, many other factors which are part and parcel of the particular disease in question. By all odds the most important is infection with its resulting fever. Fever raises the basal metabolism in proportion to the rise in temperature.[10] It seems to cause no particular change in the carbohydrate and fat metabolism. Carbohydrates are oxidized if available; if not available, fat supplies the necessary heat. The protein metabolism, however, is distinctly increased in all infections which are accompanied by that somewhat indefinite condition known as toxemia. Here we encounter the so-called toxic destruction of protein. The patient cannot be brought into nitrogen equilibrium even though we give in the diet enough calories to cover the total heat production. This phenomenon was carefully studied in 1909 by Shaffer and Coleman.[28] They administered to typhoid patients diets containing 3,000 or 4,000 calories largely in the form of carbohydrate. In doing this they eliminated from the clinical picture of typhoid fever the usual factor of partial starvation and, incidentally, they showed that most of the distressing features of typhoid were due to starvation rather than infection. By means of these large diets they were able to bring a few patients into

nitrogen equilibrium. The subsequent studies by Coleman and Du Bois[7] showed that these diets contained almost twice as many calories as the patients actually produced. It is quite obvious that there must have been some abnormal factor increasing the protein metabolism. Shaffer and Coleman also tried to ascertain the nitrogen minimum in typhoid fever and found that it was impossible even on an ample diet containing little protein to bring down the nitrogen elimination to the low levels obtained by Landergren and others in health. Kocher[16] confirmed this in paratyphoid fever. In Figure 17 I have sketched the position of the basal metab-

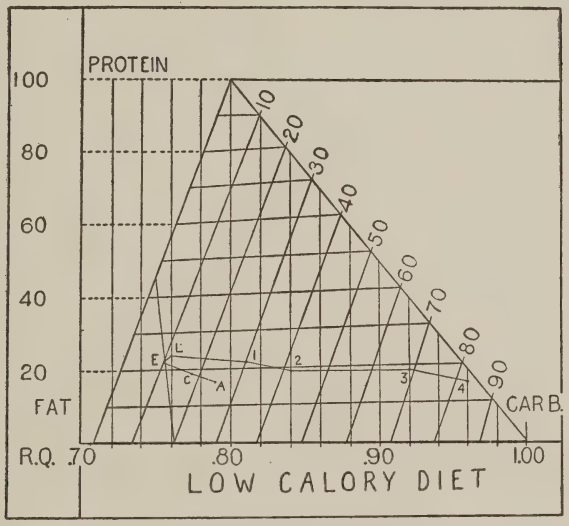

Fig. 17.—Metabolism map of typhoid patients on low calory diet: A, Ascending temperature period; C, continued temperature; E, early steep curve; L, late steep curve; 1, first week of convalescence; 2, second week of convalescence, etc.

olism during the different weeks of typhoid fever as estimated from the respiration experiments recorded in the German literature before 1912. These patients were given the old-fashioned restricted fever diet, and the respiratory quotients were measured twelve hours or more after the last meal. Carbohydrate furnished a very low percentage of calories

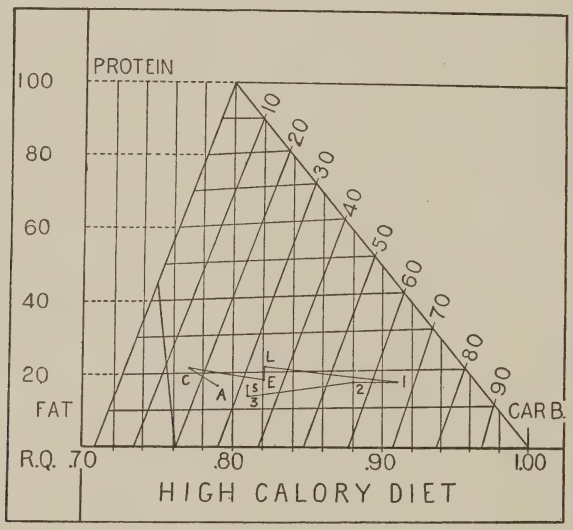

Fig. 18.—Typhoid patients on high calory diet.

during the fever. In convalescence, when the patient was consuming enormous amounts of food, the carbohydrate plethora lasted so long after the evening meal that little or no fat was metabolized.

Figure 18 shows the results obtained in the patients who were placed on the Coleman-Shaffer high calory diet. During the first part of the fever this could be administered in

large amounts, but later, as the patients' appetites improved, they were brought into about the same metabolic position that is occupied by normal men. The toxic destruction of protein was checked though not abolished, the patients were removed safely from the zone of ketosis, and the metabolic phenomena of convalescence were often clearly manifest several weeks before the febrile period ended. Some of the patients even with high temperatures were able to replenish the stores of body fat. This diet has changed the clinical picture of typhoid fever and caused a distinct reduction in the mortality.

No other fever has been studied as thoroughly as typhoid. From the limited data on hand, however, we can say that the picture is almost exactly duplicated in erysipelas and probably the other fevers with similar toxemia. Tuberculosis is a much more chronic disease than typhoid and the toxemia does not seem to be so violent. McCann and Barr[21] have shown that the normal levels of nitrogen minimum can be rather closely approximated in tuberculosis, thus proving that there is much less toxic destruction of protein in this disease. If time would permit I should like to discuss the experiments of McCann[20] on the effect of food on the pulmonary ventilation in tuberculosis. He proved that if much protein were given, the specific dynamic action would increase the respiratory exchanges and cause harmful increments in the work of the lungs. He pointed out the dangers of overfeeding and showed that the metabolism of a given number of calories in the form of fat was accompanied by a distinctly lower pulmonary ventilation than if a diet which con-

tained the same number of calories in the form of carbohydrate were administered.

So much for the factors of fever and infection. We must next turn our attention to the circulation and the kidneys. It is doubtful if we shall ever be able to find out the true level of protein metabolism in heart failure or severe renal disease. In these two conditions the urinary output is so irregular that it gives us no indication of the rate of protein metabolism. All we can do is to assume that the protein metabolism will be at least as high as in starving men. If this is the case, we can give cardiac and nephritic patients at least 4 to 6 gm. of nitrogen in the food without causing the slightest increase in the formation of urea. There does not seem to be any valid reason for the protein-free diets that have sometimes been advocated. If we really want to depress the formation of urea to the lowest possible point in kidney disease we should give a diet of ample calories containing 20 to 30 gm. of protein. We must, of course, take into consideration all the other factors of the disease, paying due attention to the gastro-intestinal tract, and so forth. In heart failure and in some cases of nephritis many clinicians employ with excellent results the diet of 1 liter of milk without any additional fluids. This is a submaintenance diet and the patient is, therefore, losing some of his body fat.

In hyperthyroidism the increase in total metabolism overshadows all the other metabolic phenomena. It has long been recognized that if we wish to keep these patients in nitrogen balance we must give a large excess of calories above the basal. Boothby and Sandiford[5] have recently

explained the reason for this apparent excess. They have shown that in hyperthyroidism a given amount of work requires about twice the expenditure of calories that it does in normal individuals. They have found no evidence of abnormal protein metabolism. Rudinger[25] in studying two young patients demonstrated only a slight increase in the nitrogen minimum. More experiments on this minimum are needed badly. The carbohydrate metabolism in hyperthyroidism is supposed to be abnormal, but I am not familiar with any definite proof of such abnormality. One of our patients[9] in the Sage calorimeter, in 1914, showed a glycosuria, yet after taking 100 gm. of glucose was able to derive 76 per cent of his calories from this source, his respiratory quotient being 0.943. Sanger and Hun[26] have recently noted the same phenomenon. Cramer,[8] as a result of his work on rats, advanced the hypothesis that in hyperthyroidism the liver could not store glycogen. In 1916 we had an unusual opportunity of studying glycogen storage in a young man with exophthalmic goiter who fasted for three days during which time he was observed at intervals in the calorimeter. His respiratory quotient fell at almost exactly the same rate as Benedict's fasting subject, Levanzin. It was possible to calculate that he oxidized in the first three days between 300 and 360 gm. of carbohydrate, which is well within the normal limits. This result has been confirmed recently by Richardson and Levene.

The studies of recent years have been of great service in removing many of the mysteries that enveloped the metabolism in disease. Our subject has become much simpler than

it was ten years ago. We have reached the point where by means of diet we can usually modify the patient's metabolism and accomplish certain specific results. These results, however, can be attained only by careful study of the various processes which are taking place within the patient's body.

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MUSCULAR ACTIVITY AND CARBOHYDRATE METABOLISM

Archibald Vivian Hill

THE PROBLEM: INTRODUCTION

It has long been discussed whether the breakdown of carbohydrate, rather than of other substances, is primarily responsible for the provision of energy in muscular contraction. It is known and accepted that work may be done, in the general melting-pot of the body, by the use of any kind of foodstuff. We are now concerned, however, specifically with the *primary* process of muscular contraction. In the complete chain of processes involved in long-continued exercise, this primary process may be disguised, or even apparently obliterated, by simultaneous transformations which take place between the different food constituents. Considering the internal combustion engine, it is obvious that petrol and benzole may be used indiscriminately for providing power and driving the machinery. In the same way, however, as we ask whether carbohydrate is the specific fuel of muscle, or whether fat may be used in an identical manner, so we might query whether petrol or coal can be used in an internal combustion engine. The obvious answer is that coal must be prepared beforehand by distillation, before it can be used in the engine, while petrol can be used

directly; and that in the preparation of coal to form benzole for use in the engine, a considerable proportion of the energy of the coal is wasted, as regards its work-producing power. Putting our problem in terms of the modern theory of muscular activity and assuming that the initial process in contraction—that which causes the mechanical response—is an entirely non-oxidative one, consisting of the formation of lactic acid from glycogen, we are asking now whether the recovery process by which the lactic acid is restored to its precursor can go on at the expense of *any* oxidation, or *only* of that of carbohydrate. *May the recovery mechanism, so to speak, be driven by any kind of combustion, as a steam engine may be, or is it necessary specifically to combust carbohydrate?*

THE RESPIRATORY QUOTIENT

It has long been known that the respiratory quotient during prolonged steady exercise is not unity. It varies with the diet. That, however, does not answer our question. Carbohydrate may be used exclusively in the muscular process of breakdown and recovery, but as fast, or almost as fast, as it is used up it may be restored, in the general metabolism of the body, by the breakdown of some other substance, for example, of fat. The combustion of carbohydrate, followed by the reformation of carbohydrate from fat, would affect the respiratory quotient in a manner exactly similar to the direct combustion of fat.

MECHANICAL EFFICIENCY ON DIFFERENT DIETS

The beautiful and very convincing experiments of Krogh and Lindhard, published in 1920, showed with little pos-

sibility of doubt that the combustion of carbohydrate has some special connection with muscular activity. Employing a method where the respiratory quotient may be determined with an average error of only ± 0.002, measuring the cost of doing a given amount of work of moderate intensity in a highly trained and carefully observed and calibrated subject, and varying the substances metabolized by varying the diet during and before the experiment, they found the cost of work (that is, the total amount of energy used in doing a given amount of work) to be a linear function of the respiratory quotient, falling as the respiratory quotient rose. Of the total energy used in any effort, granting that the respiratory quotient be correctly measured, the fraction which is derived from fat may be shown to be a linear function of the respiratory quotient. I say intentionally, *if the respiratory quotient be correctly measured;* we will discuss later the variations of respiratory quotient produced by lactic acid in the body during and after severe muscular work. Such variations, however, do not affect Krogh and Lindhard's experiments, in which the exercise was moderate, requiring only about 1 liter of oxygen each minute, and continued for a long time. If now we assume that carbohydrate oxidized is utilized directly for work, or (more accurately) for recovery from work and the fat only after "conversion" involving metabolic processes and loss of energy, the cost of work should be a linear function of the respiratory quotient—as Krogh and Lindhard found. As the mean of a long, careful, and carefully weighted series of observations, which give one all the impression of extreme reliability,

they found, assuming carbohydrate to be utilized directly for the production of work (or, as I should rather say, for carrying on the recovery process) that fat may be so used only after "conversion" involving a 10 per cent loss of energy; in modern terms, the recovery process is 10 per cent less efficient when fat is oxidized than when carbohydrate is oxidized. This suggests strongly that the primary breakdown is of carbohydrate, and that fat is used only in a secondary manner, for example, to restore the carbohydrate which has disappeared. These experiments of Krogh and Lindhard are particularly valuable since they were made on intact animals, namely, healthy men, and involved the complete process in the whole mechanism.

LACTIC ACID

The most important line of evidence in this connection starts from the work of Fletcher and of Fletcher and Hopkins, leading to that of Meyerhof, Embden, and others. The phenomena of muscular fatigue are known to all, both personally and in the laboratory, as also is the effect of oxygen thereon. An isolated muscle stimulated in nitrogen soon fatigues and never recovers: an isolated muscle stimulated in oxygen may go on contracting for days. These observations of Fletcher led to the lactic acid story. In an isolated muscle at rest and without oxygen the acid accumulates slowly, faster at a higher temperature; with a sufficient supply of oxygen it remains at a low value. Stimulation also will produce lactic acid; in oxygen this lactic acid is removed. According to Embden and his co-workers, the

origin of this lactic acid in the muscle is a hexose di-phosphoric ester. They succeeded in isolating an osazone similar to that described by Harden and Young in the case of yeast. This hexose phosphate is presumably a very unstable substance; it has not been isolated from muscle; its amount can be estimated only indirectly and on certain assumptions. Embden regards it as the immediate precursor of the lactic acid which appears, though Meyerhof's experiments make it clear that it is glycogen which bears a quantitative relation to lactic acid. The amount of Embden's "lactacidogen" present in a muscle at any moment must be estimated by measuring the inorganic P_2O_5 immediately after, and one or two hours after, the fine division of the muscle: the increase in the P_2O_5 is supposed to represent the "lactacidogen" which has broken down. The evidence, though indirect, appears to yield results so definite that it is difficult not to believe that hexose phosphate is somehow intimately concerned with muscular activity. After severe muscular work, following a dose of phloridzin in rabbits, and after strychnine convulsions in rabbits and in dogs, there is a marked diminution in the "lactacidogen" present in their muscles. It is interesting too to record that, according to a communication of Robison and Kaye to the British Biochemical Society, the injection of insulin causes an increase in the "lactacidogen" of muscle. It must be admitted, however, that the rôle of the hexose-phosphate is not yet clear.

THE CARBOHYDRATE ORIGIN OF LACTIC ACID

It is very natural to attribute a carbohydrate origin to the lactic acid which is concerned so intimately with muscular contraction. By the fermentation of various types of carbohydrate lactic acid may be formed, and Meyerhof has shown by a series of direct experiments, confirmed by independent methods at Cambridge by Foster and Moyle that when lactic acid appears in muscle, whether from anaërobic conditions or from fatigue, an equivalent amount of glycogen disappears; in the converse process of recovery when the lactic acid is removed, glycogen reappears, not this time in equivalent amount, but with a 25 per cent loss, which is accounted for by the oxygen used and the heat produced in the recovery process. In the isolated muscle, therefore, there can be no doubt that lactic acid has a carbohydrate origin and is restored to carbohydrate in recovery, a fraction of it only being used in the oxidative processes required to drive the recovery mechanism. That this recovery reversal of the glycogen-lactic acid breakdown is, at any rate in isolated muscle, carried out at the expense of energy derived from carbohydrate oxidation, is made the more certain by Meyerhof's observation that *the respiratory quotient of recovery is unity*. Moreover, in the isolated muscle there is no sign of any diminution in the fat contained in the muscle, as was shown by Winfield and confirmed to some degree by later and more severe experiments at Manchester (unpublished). The total amount of glycogen present in a muscle is adequate to account for the whole of the energy used in the most prolonged series of contractions that that

muscle is capable of carrying out in oxygen, even under the most favorable conditions. It is possible, of course, that no transformation or combustion of fat is possible without the co-operation of other organs (for example, the liver) or of the body as a whole. We shall see later how far this objection applies. In the isolated muscle, however, we may safely assert that the only processes which are known to occur, the formation of lactic acid from glycogen in the initial phase and the removal of the lactic acid, coupled with the oxidation of a small amount of it in the recovery phase, involve nothing but reactions with, and by, carbohydrate.

PANCREATIC CONTROL

Azuma and Hartree have shown that insulin has no effect whatever on the recovery oxidation in isolated muscle, and Foster and Woodrow that it has no effect on the lactic acid formation in resting surviving muscles. In intact animals under insulin treatment glycogen tends to disappear from the muscles (Dudley and Marrian), possibly partly to form a hexose phosphate, certainly not to form lactic acid. Moreover, Himwich, Loebel, and Barr have found that lactic acid formation in the diabetic individual is just as much the basis of muscular contraction as in the normal. This has been confirmed independently by my colleagues Long, Lupton, and Hetzel (hitherto unpublished), not only in the case of the formation of lactic acid, but in that of its removal in recovery. Apparently lactic acid is just as much involved in the mechanism of contraction in the diabetic as in the normal man. That there is, however, some factor in the pancreas

concerned in the carbohydrate metabolism of muscle was shown by preliminary observations of Hopkins and Winfield in 1915, who found that pancreas preparations have an inhibitory action on the formation of lactic acid in minced muscle. Apparently in the pancreas there is a substance, stable at high temperatures, which has a controlling action on the carbohydrate breakdown of muscle. This substance is not a ferment, and may be present in commercial pancreas preparations several years old. Foster and Woodrow followed up this clue and established the fact that there is an inhibitory agent for the anaërobic lactic acid formation in muscle which may be isolated from the pancreas and produces considerable inhibition even under conditions leading usually to the maximal lactic acid formation. This substance is not insulin, which has no such effect. They suggest that this new unknown substance has a specific controlling function on the carbohydrate metabolism of muscle, and that carbohydrate metabolism may be grouped into two great subdivisions, that of the body as a whole under the control of insulin, and that of muscle, to some degree under the control of this new pancreatic hormone.

ANALOGOUS METABOLISM IN OTHER CELLS

An interesting side-line from Foster and Woodrow's experiments arises when we remember that, according to the modern view of muscle, the basal metabolism of the intact animal is in large part the recovery from the resting lactic acid production of its muscles. This new pancreatic hormone might be expected, therefore, to control the basal

metabolism, and possibly we may find in it a means of antagonizing an overactivity of the thyroid—though that is guesswork. Foster and Woodrow, on the basis of these experiments, have put forward the theory of carbohydrate metabolism (not referred to) to which, on quite other grounds, those who have been working on muscular activity have been inevitably reduced, namely, that the carbohydrate metabolism of muscle is a different thing from that of the body as a whole. That this carbohydrate metabolism of muscle, however, involving lactic acid is not unique is indicated by many lines of evidence. For example, Stephenson and Whetham have found that *Bacillus coli*, in a medium containing glucose, uses oxygen if it can get any and produces CO_2 and lactic acid; in nitrogen, CO_2 and lactic acid are produced up to a certain limit, the fermentation being a self-inhibited one; in oxygen less lactic acid is produced, more CO_2 is liberated, and more oxygen is used. Apparently these organisms, in the presence of sufficient oxygen, can break down glucose completely to CO_2 and water. In the absence of sufficient oxygen they break it down, as does muscle, to lactic acid. If suspended in a medium containing no glucose but ammonium lactate, in the absence of oxygen they can do nothing; in the presence of oxygen they can produce CO_2 and use up oxygen, as does a muscle carrying out its recovery process.

Again, Warburg, using the delicate gas-manometer method of Barcroft, has measured the CO_2 produced and the oxygen used by various tissues suspended in a glucose-Ringer solution. Some of the CO_2 is produced by combustion of carbo-

hydrate, some is driven out from preformed bicarbonate by acid formation. Expressing as "extra CO_2" the amount of CO_2 produced in excess of that derived from the oxygen used in burning carbohydrate, the "extra CO_2" is a measure of the lactic acid produced by the fermentation of glucose, that is, of the carbohydrate broken down, while the oxygen used is a measure of the carbohydrate oxidized. Normal tissues give a ratio, (extra CO_2)/O_2, of practically zero. Cancerous tissues from a rat, however, give an average ratio of 3.6; human cancerous tissues give a ratio usually from 2 to 4, but varying over wide limits. In normal tissues, therefore, the oxidative process of glucose metabolism is effective and the fermentative process is small; in cancerous tissues the oxidative process is ineffective and the fermentative process is large. If Warburg is correct, the cancerous tissue *is like a muscle in which the recovery mechanism has almost broken down.*

GLYOXALASE

In all tissues of the body except the pancreas Dakin and Dudley found a ferment, glyoxalase, which is capable of transforming methyl glyoxyl, $CH_3.CO.CHO$, into lactic acid, $CH_3.CHOH.COOH$. This ferment is inhibited by excessive acidity, that is, by an accumulation of the product of its own activity. It is present also in the blood of diabetic persons and in the blood and liver of diabetic dogs. An extract of the pancreas inhibits this reaction, and Dakin and Dudley have called this inhibiting substance *antiglyoxalase.* Antiglyoxalase is destroyed by heat, and on other

grounds also it would appear not to be the same as the pancreatic hormone of Hopkins and Winfield, and of Foster and Woodrow. It is conceivable that the normal path of carbohydrate metabolism may lie through the formation of lactic acid by glyoxalase from methyl glyoxal; unfortunately, except for the presence of this potent enzyme, glyoxalase, there is little evidence either for or against this theory.

THE FATE OF LACTIC ACID IN RECOVERY

The fate of lactic acid, which is an undoubted intermediary in the breakdown of carbohydrate in the muscle, was long debated. Its removal during recovery, as established by Fletcher and Hopkins, was naturally credited at first to a simple process of oxidation. There were, however, certain fundamental difficulties about this to which there seemed to be no answer. The heat of combustion of glycogen, from which it is formed, is, according to Slater, 3,836 calories for each gram, when the glycogen is in its fully hydrated form, as it occurs in solution in the muscle; that of dissolved lactic acid is 3,601 calories (Meyerhof), leaving a total energy for the transformation of the one to the other of only 235 calories. This small quantity then is the total energy available in the initial transformation of glycogen to lactic acid, while in the complete process, if the lactic acid were then oxidized, 3,836 calories would be liberated. The mechanical efficiency, therefore, of muscular contraction, supposing *the whole* of the initial energy were turned into work, could not exceed 6 per cent. Values of 25 per cent have been found in the case of man. Actually the initial liberation of heat[2]

for each gram of lactic acid formed in muscle is larger than 235 calories, being about 296 calories. The difference has been attributed by Meyerhof to the neutralization of the acid by buffered alkaline protein salts inside the muscle fiber. There is no doubt that the acid is neutralized as soon as it is formed, since the hydrogen-ion concentration does not rise appreciably. Moreover, there is not enough phosphate or bicarbonate present in the muscle to neutralize all

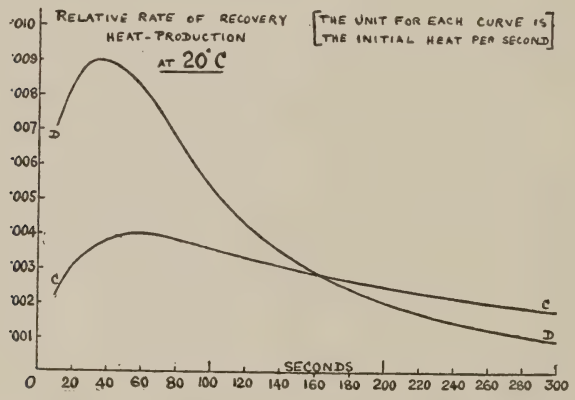

Fig. 19.—Delayed heat production of an isolated muscle in the presence of oxygen: C 0.03 sec. and D 0.20 sec. tetanus. (Hartree and Hill, 1922.)

the lactic acid formed. Neutralization by phosphate and bicarbonate liberates only a little heat, while that by buffered protein salts liberates a large amount. Even assuming, however, that 296 calories are liberated in the initial phase and that the whole of this energy is turned into work, if the lactic acid were then oxidized the efficiency could still be only 8 per cent. Clearly the lactic acid is not oxidized.

It has proved possible, moreover, to measure the total

amount of heat liberated in the recovery process (Fig. 19), which in the latest and most careful measurements has come out almost exactly equal to the total heat liberated in the anaërobic phase. Thus, in the formation of and the subsequent removal of a gram of lactic acid only 740 calories of heat are found, which is only about one-fifth of what would occur if the lactic acid once formed from glycogen were subsequently oxidized *in toto*. Apparently, of every 5 molecules of lactic acid removed in the recovery process only one is oxidized; the remaining four are restored to the place, or as the substance, from which they arose. There is no possibility of any error in the general conclusion to be drawn from these heat measurements: the difference to be explained is far too large; probably indeed the heat measurements provide us with the most accurate means of determining the "efficiency of recovery," as we may call it (or, in Meyerhof's term, the "oxidative quotient"), that is, the ratio of the amount of lactic acid removed to that oxidized in its removal. The best value to assume seems to be, in frog's muscle, about 5 : 1, and this is confirmed, as I shall show later, by two independent lines of experiment on man. This conception of the fate of lactic acid has been confirmed by Meyerhof's direct observations of the glycogen restored and the lactic acid lost during the recovery process. All lines of experiment, therefore, on the isolated muscle indicate about the same value for the efficiency of recovery. There is no doubt that one must regard lactic acid in muscle as being not so much the fuel as part of the machinery.

THE RECOVERY PROCESS

Consider now the recovery process in further detail. In the whole animal, without special precautions which we shall discuss later, it is not easy to isolate the recovery process from other events in the animal at large. In the isolated muscle the chemical method of investigation is not sufficiently analogous to what happens during normal existence, since the oxygen supply is cut off from its normal route by the cessation of the circulation, and (having to depend upon diffusion) is necessarily inadequate. The *oxidative removal of lactic acid in isolated muscles stimulated to severe fatigue has to take place under conditions of severe oxygen want, and is a very protracted affair.* The speeds, for example, at different temperatures cannot be compared, since they depend simply on the rate at which oxygen can pass in by diffusion from outside. Fortunately, another method is available which, compared with the chemical method, is of surpassing sensitivity, namely, that in which the heat production is measured.[10] Myothermic technic is so sensitive and so well under control that it is possible to measure and to analyze the course of the heat production for many minutes after only a single twitch of the muscle, in which case the total amount of energy involved and the total amount of oxygen used are so small that the amount of the latter originally dissolved in the fluid of the muscle is more than adequate to account for the whole of the oxidation carried out. We are independent, therefore, of the oxygen supply, and can study the speed and magnitude of the recovery process in a muscle provided with an entirely adequate amount of oxygen.

We find, when we stimulate a muscle, that there is initially a large production of heat, which must be attributed to the formation of lactic acid from glycogen and its subsequent neutralization. Then commences a slow process of recovery, in which heat is liberated continuously for many minutes until the muscle has been completely restored to its initial condition. The heat production rises rapidly at first, attains its maximum in a few seconds to half a minute, and then slowly falls to zero again, along a curve which is roughly exponential. This curve of recovery heat production is the thermodynamic outline of the recovery process, into which fuller details must be drawn later by biochemical analysis. The speed of the process depends on temperature: it is increased very largely by a rise of temperature, decreased by a fall, so that in a frog at 0° C. complete recovery, even from a few hops, must take an hour or more! Extrapolating the results on frog's muscle to the temperature of the human body, the recovery process from moderate effort should be nearly complete in two to three minutes, given an adequate supply of oxygen, as, indeed, we find it to be. Its speed depends also on the size of the initial breakdown of which it is the result, not only absolutely but relatively. Its speed is affected by the hydrogen-ion concentration, being diminished by a rise and increased, up to a certain limit, by a fall, beyond which, however, it remains constant. Carbon dioxid, in concentrations of 10 to 15 per cent, produces a considerable fall in the rate of the recovery process, working much more quickly than do other acids, presumably because CO_2 can more easily penetrate the muscle-fiber. The effect

of hydrogen-ion concentration on the speed of the recovery oxidation is analogous to that on the speed of autoxidation of glutathione or cystein. The total extra amount of heat liberated by oxidation in the recovery process is almost exactly equal to that set free in the anaërobic breakdown alone.

THE "ACCUMULATOR FUNCTION" OF MUSCLE

These facts have led us to the conception of the muscular machine as an accumulator of energy, analogous in its way to a lead electrical accumulator. The initial discharge, which may take place at a high rate, depends in no way on the oxygen supply; the final recharge, which is slower, depends directly on oxidation. In voluntary muscle all oxidation must be regarded as recovery oxidation: even though oxidation takes place during continuous exercise, and appears to be contemporary with the exercise, it must really be regarded as recovery from previous elements of the exercise.

It is probably not true to assert that in all organs and tissues oxidation is recovery oxidation. For example, Starling and Verney have recently shown that in a kidney secreting normally the administration of KCN, which abolishes oxidation, produces immediately a change in the secretion, making it in all respects similar to a filtrate from the blood. Apparently "knocking out" oxidation immediately "knocks out" the capacity of the tubule cells to perform their normal function. It is probable that the same immediate dependence on oxidation exists in other tissues. Possibly those

organs, in which sudden and violent activity may be required at a moment's notice and which are stimulated to activity through nerves, tend to act, as does voluntary muscle, like an accumulator; while slower tissues, in which rapid and violent response is not so necessary, may be content to remain dependent for their energy on oxidation, as does an internal combustion engine.

THE RECOVERY PROCESS IN MAN

The conception that all muscular oxidation is really recovery oxidation has produced an extensive change in outlook in regard to respiratory experiments on man. One of the fundamental difficulties of a large animal is the supply of oxygen to his tissues. When muscular exercise starts the oxygen intake rises, attaining a maximum in man in two to three minutes. Respiration and circulation have to be worked up and the recovery process has to get under way. Muscles, however, are required for immediate and violent use, and even the maximal intake of oxygen, which in athletic men is about 4 liters per minute, can provide energy only for comparatively moderate exercise; in order to attain even that maximum a period of two to three minutes is necessary. Actually the human body is capable of exerting itself nearly ten times as violently as it could possibly do were it obliged to obtain all its energy immediately by combustion. Just as a lead storage cell is found to accumulate sulphuric acid in the plates during its activity, so a muscle is found to accumulate lactic acid; just as the storage cell has its sulphuric acid removed from plates to solution dur-

ing recharging, so the muscle has its lactic acid restored to its precursor in recovery. With this conception it is of interest to study the process of recovery not only in isolated muscles, but in man, and in the last few years this study has proceeded a considerable way, especially by the efforts of my colleagues, Long and Lupton. Lupton, alas, has not

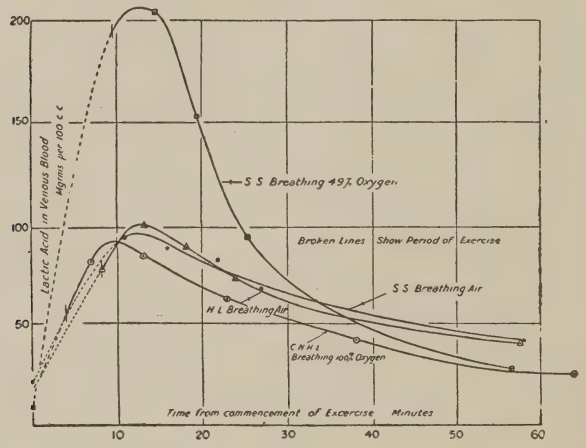

Fig. 20.—Lactic acid in human blood after severe muscular exercise; two experiments in air, one in 49 per cent oxygen, one in 100 per cent oxygen. Note that the recovery process is not quite complete at the end of the time shown in the diagram. (Hill, Long, and Lupton, 1924.)

lived to reap the reward of his devoted work, or to realize the full importance of what he did.

LACTIC ACID IN MAN

Lactic acid may be studied directly in man by its estimation in blood removed in the usual way from a vein. During muscular activity the lactic acid in the blood rises, attaining

finally, if the exercise be continued long enough, a maximum characteristic of the effort made. After a while the lactic acid distributes itself by diffusion equally in all tissues which are directly in contact with the blood-stream. During recovery this lactic acid disappears, in a period depending on the severity and duration of the preceding exercise, but not exceeding in normal man about ninety minutes (Fig. 20). The removal of lactic acid from the blood, which is a sign of its preceding removal from the muscle, is produced by oxidative processes occurring in the latter. These oxidations can be studied by ordinary respiratory methods, employing the Douglas bag technic. The initial phase of the recovery process, which is rapid and is concerned with the oxidative removal of the acid in the muscles where it was formed, can be followed by means of collections in a series of bags. The recovery oxidation falls rapidly, and after moderate exercise reaches zero in a few minutes. If, however, the exercise was severe, the lactic acid will have had time to escape from the muscles into the blood, and into other tissues in contact with the blood, and a second phase of recovery will occur, the removal of lactic acid which has escaped. This second phase may be very protracted and last as long as eighty minutes. The total oxygen used in the recovery process in this way we have named[13] the "*oxygen debt at the end of exercise.*" Assuming, what may be shown to be very nearly true, that it is all used in the oxidative removal of lactic acid, and employing a value of 5 : 2 : 1 for the efficiency of recovery, we may calculate from the oxygen debt the lactic acid present in the body at the end of exercise. We find

that 3 gm. or more of lactic acid may be liberated each second in the muscles of a powerful man, and that the body is able to tolerate an amount up to a *total of 130 gm.* The oxygen debt may attain a value of 18.7 liters!

This lactic acid formation, therefore, in the human body is not a small or unimportant factor in muscular exercise; it is the keystone of the whole structure and has a large, indeed, a preponderant, effect on the respiratory quotient.[12]

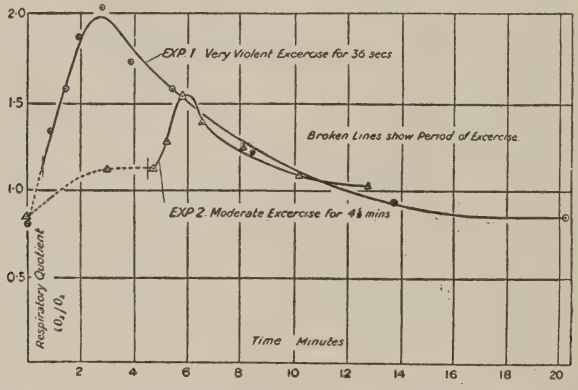

Fig. 21.—The respiratory quotient during and after muscular exercise. These figures show the initial phase of recovery only. The final phase is shown in Fig. 22. (Hill, Long, and Lupton, 1924.)

The respiratory quotient varies in a striking manner, up and down, during the onset of severe exercise and in recovery from it. At first it rises (Fig. 21), attaining a value up to 2, during and immediately after the phase of lactic acid liberation, and while the respiratory center is still endeavoring to cope with the increased hydrogen-ion concentration of the tissues. Before the hydrogen-ion concentration of the body can have returned to its previous resting value an

amount of CO_2 must be driven off equivalent to the lactic acid still present. After this previous level of the hydrogen-ion concentration has been attained, which happens several minutes after recovery has commenced, the lactic acid continues to decrease and CO_2 has to be retained by the body, since otherwise the latter would become far more alkaline than previously. In the later stages of recovery the CO_2

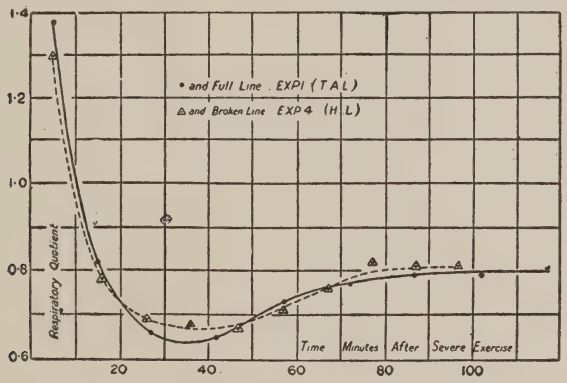

Fig. 22.—The respiratory quotient after severe muscular exercise. Note that in the later phases, while carbon dioxide is being retained to compensate for that initially driven off, the respiratory quotient falls to a very low level, returning to its final value at about eighty minutes. (Hill, Long, and Lupton, 1924.)

retained is a measure of the lactic acid removed and very low values of the respiratory quotient may be found, down to 0.6 (Fig. 22). Assuming that CO_2 retention to be a measure of the lactic acid removed, and the oxidation in excess of the basal value a measure of the lactic acid oxidized, we may determine in man the efficiency of recovery by respiratory methods, and its value comes to about 5 : 1, the same as in isolated muscle. Another method may be used in

estimating the same quantity in man. If the lactic acid found in blood be assumed, in the later stages of recovery, to be uniformly distributed in all the soft tissues of the body which are in immediate contact with the blood-stream, we may calculate by two observations of the blood over any interval the total amount of lactic acid removed, and from the excess oxygen used in that interval we may again determine the ratio of lactic acid removed to lactic acid oxidized. We find as before a value of about 5 : 1, so that in the complete and intact animal the mechanism of recovery appears to be the same as in the simple isolated muscle.

THE USE OF THE RESPIRATORY QUOTIENT

These large variations in the respiratory quotient, during severe exercise and in recovery therefrom, show how necessary it is to exercise the greatest possible precautions if we wish to draw any conclusions from the respiratory quotient as to the substance being oxidized. Such precautions were taken in the experiments of Krogh and Lindhard. The exercise must be moderate and very long continued, and the whole condition of the subject must be "steady." Then only are deductions reliable; *otherwise the value of the respiratory quotient tells us more about the fluctuations of lactic acid in the body than about the nature of the metabolism.*

THE OXYGEN "REQUIREMENT" OF EXERCISE

When muscular exercise commences the oxygen intake rises to a value which is either the equivalent of the exercise, if the latter be moderate, or is the maximum character-

istic of the individual subject, if the exercise be severe. In the latter case the exercise can be continued only for a time, the lactic acid accumulates, fatigue comes on, and the muscles finally are incapable of further effort. The oxygen intake is a measure of the severity of the exercise *only* if the latter is sufficiently protracted to enable a steady state to be attained, and sufficiently gentle to ensure that there is not a constant accumulation of acid leading to an oxygen debt. Hence by a study of the oxygen intake and the CO_2 output we can never really determine the nature of the primary oxidations of muscular activity, since the exertion must be continued for a long time until the body and all its processes are in a steady state, and the primary reactions of muscular recovery may then be masked by other and secondary effects. The oxidation of carbohydrate required to drive the recovery process may be confused, for example, with the re-formation of carbohydrate from fat.

This fact and others have led us to a study of what we call the "oxygen requirement." The subject of the experiment takes exercise of any character and of any duration, the total oxygen used during the exercise and in complete recovery from it being measured. An initial and a final estimate of the resting oxygen consumption give us a base line from which the total oxygen consumption resulting from the exercise, during and in recovery from it, may be calculated. The measurement of the oxygen requirement is valuable, since it can be made in the case of any type of exercise, for example, walking up a single flight of stairs, or in very violent exercise which could not be continued long

enough to make a measurement of the oxygen intake possible or useful. It may be a valuable criterion of the mechanical efficiency of work, etc. The oxygen requirement for a short element of exercise is always a measure of the total amount of energy required by the body for that exercise, assuming, as we shall see below, the energy value for oxygen corresponding to the oxidation of carbohydrate.

THE RESPIRATORY QUOTIENT OF EXERCISE AND RECOVERY

Much greater interest attaches to the respiratory quotient when we consider not only exercise, but subsequent recovery. Taking the case of a small element of muscular exercise, such as running slowly for thirty seconds, the resting respiratory exchanges are measured carefully, both before the exercise and after complete recovery. The expired gases are collected both throughout the exercise, and during a recovery interval sufficiently long to ensure that the metabolism has returned absolutely to its initial state. The *excess* oxygen used as a result of the exercise and the *excess* CO_2 given out are then determined by analysis and calculation: *they are found to be precisely equal.* The same is true of fairly violent exercise for a short interval. In the case, however, of very violent exercise, the recovery process may be very protracted and the respiratory quotient of the excess metabolism may be less than unity. If the oxygen requirement of a very long period of exercise be measured, it is obvious that the respiratory quotient will not be unity. In such a case nearly all the excess of oxygen used and of CO_2 produced by the exercise occurs during the latter, while

the respiratory quotient is, say, 0.85. Thus, as we should expect, when a bout of exercise is increased in duration from very short to very long, the respiratory quotient of the complete cycle passes gradually, from a value of unity for the very short, to a lower value characteristic, as in the experiments of Krogh and Lindhard, of prolonged steady exercise. These results appeared first incidentally in a study of the oxygen requirement of exercise, carried out for another purpose. We noted, however, that of about twenty experiments practically all gave a respiratory quotient of the excess metabolism of about unity, the mean value being 1.03. The small excess we attributed to the fact that in these experiments recovery was not quite complete, and the small CO_2 retention of the last phase had not come within our observation. Since then my colleague, Dr. Furusawa, has examined the matter more carefully. His experiments (Tables 1 and 2) show that the respiratory quotient of the excess metabolism due to a *short element* of muscular exercise is unity. This is the case even if the subject, having lived for several days on a diet of fat and protein, has a resting respiratory quotient of little more than 0.71 (Table 2). If the exercise be prolonged the stores of carbohydrate are used up and have to be reformed by the transformation of other substances, presumably of fat; such a transformation acts on the respiratory quotient just as though fat itself were being oxidized, so that the respiratory quotient falls. Given, however, an element of muscular exercise, so moderate in duration and severity as to produce no measurable carbohydrate lack in the muscles and no disturbance

TABLE 1

(Unpublished experiments by K. Furusawa)

NORMAL DIET

R. Q. of Excess Metabolism Due to Exercise

Duration of exercise, minutes.	Steps per minute.	Duration of collection, minutes.	Excess metabolism, CO_2/O_2.	R. Q.
0.5	92	10	355/350	1.00
0.6	64	10	485/490	0.99
1.0	146	20	2120/2037	1.04
1.0	160	31	4185/4048	1.03
2.0	208	76	11230/10251	1.09
10	120	60	8720/8900	0.98
12	160	85	19860/18620	1.06
15	146	77	25670/25960	0.99
20	146	110	41310/42210	0.98
28	146	63	49520/52900	0.94
30	120	70	20225/20345	0.99
30	146	105	49420/56230	0.88

Not postabsorptive, but several hours after a meal.

Average R. Q. resting, 0.85

Average R. Q. excess metabolism = 1.02

(Up to 30 liters O_2.)

of metabolism in the rest of the body, we find that *the whole oxidative cycle of recovery is carried out at the expense of carbohydrate.*

This confirms and amplifies the experiments of Krogh and Lindhard with which we started this discussion, and makes clear how the experiments on the isolated muscle, with their indication that carbohydrate is the essential fuel of muscle, may be reconciled with the respiratory quotients obtaining in prolonged moderate exercise in man. *The primary fuel of muscle is carbohydrate; the essential element in the machinery is lactic acid, itself derived from carbohy-*

TABLE 2

(Unpublished experiments by K. Furusawa)

FAT DIET

(Bacon, Butter, Milk, Fat Chops)

R. Q. of Excess Metabolism Due to Exercise

Subject.	Duration of exercise, minutes.	Steps per minute.	Duration of collection, minutes.	Excess metabolism, CO_2/O_2.	R. Q.	Compare R. Q. at rest.
K. F.	0.33	272	22	2733/2570	1.06	0.72
K. F.	1.0	146	20	2400/2260	1.06	0.71
K. F.	2.0	146	30	3020/2964	1.02	0.75
K. F.	4.0	146	43	10525/10822	0.97	0.78
K. F.	7.0	146	50	13730/15145	0.91	0.72
K. F.	9.0	146	100	18910/19940	0.95	0.76
K. F.	9.0	146	88	18835/20420	0.92	0.77
J. L. P.	0.4	216	22	3260/3341	0.98	0.77
J. L. P.	0.5	196	25	2132/1966	1.08	0.77
J. L. P.	1.0	146	26	3300/3345	0.99	0.73
J. L. P.	1.0	162	24	5255/5256	1.00	0.75
J. L. P.	5.0	146	35	12910/13775	0.94	0.75

Fatty diet, K. F. five days, J. L. P. three days, before experiments.

drate. The breakdown of carbohydrate in muscle is associated with the presence of phosphates, possibly in the form of a hexose diphosphoric ester. What the further details of the process are we do not know, but it is difficult not to believe that the utilization of fat by muscles can occur only after its previous "conversion" somewhere in the body. Even a subject suffering from severe carbohydrate want (Table 2) will oxidize carbohydrate, and carbohydrate alone, in the complete cycle of reactions resulting from an "element" of muscular exercise.

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OUR PRESENT KNOWLEDGE OF THE VITAMINS*

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In presenting the position of our knowledge of the vitamins, one is required to tell what we know of a class of substances of unknown nature which are widely distributed in our foodstuffs, and whose physiologic function we do not understand. We do not know whether the vitamins are essential, in certain cases at least, for the general biologic processes of cell activity, or whether they exert their action on cells of specific types. The methods of study of the distribution, chemical nature, and mode of action of the vitamins parallel in many respects those applicable to the study of the hormones. Indeed, the phenomena relating to the vitamins are so suggestive of the hormones that the term "food hormones" has been used by some as synonymous with vitamins. In either field of inquiry we recognize the existence of a substance which is indispensable for normal metabolism by observing what happens when it is not present. With certain of the hormones it is possible to study the effects of an excessive amount of a product elaborated by a particular tissue. In the field of vitamin study no one has demonstrated as yet any effects of administering large amounts of the active substances.

* A more extended discussion will be found in the third edition of The Newer Knowledge of Nutrition.

It is now twenty-six years since it was clearly demonstrated by Eijkman, Grijns, Hulshoff-Pol and Schaumann, working in Batavia and Java, that beri-beri was caused by lack of something in the diet which could not be supplied by polished rice, but which was present in relative abundance in the polishings of rice and in "katjang hidjoe" bean. Ten years later Funk (1913–1915) repeated and confirmed the observations of the Dutch investigators. He suggested the name "vitamine" to designate a class of substances which he believed to be necessary for the prevention of beri-beri, scurvy, rickets, pellagra, and sprue, respectively, as well as of another or others necessary for growth as distinguished from maintenance. Funk's investigations created a new interest in studies of nutrition. At that time it was only possible to demonstrate either a preventive or curative action of a vitamin preparation in the case of beri-beri, or polyneuritis, as it is called when produced experimentally in an animal. The substance which is active in the restoration of a pigeon suffering from polyneuritis is now known as vitamin B. From this beginning progress has been steady, until today almost all investigators believe the evidence is conclusive that for one or another species of animal five vitamins are necessary. Besides these principles, which have been designated as vitamins A, B, C, D, and E, another substance called bios is believed to exist, which has been confused with vitamin B.

VITAMIN A

David Livingstone, in describing the hardships endured in his explorations in Africa, called attention as far back as

1857 to a peculiar eye condition from which his party suffered while restricted to a diet of sugarless coffee, manioc roots, and meal, and said, "the eyes become affected as in the case of animals fed in experiment on pure gluten or starch." This appears to be the earliest mention of the ophthalmia which is the most characteristic pathologic change which results from a lack of the vitamin A in the diet. I have been unable to discover a description of the experiments to which Livingstone refers. Although ophthalmologists have associated for many years keratomalacia with faulty diet, as, for example, among the Russian peasants during religious fasts, the first definitely to associate xerophthalmia in the human subject with faulty diet was Mori (1904). He described about 1,500 cases of xerophthalmia or keratomalacia among children in Japan between the ages of two and five years from 1905 to 1907 inclusive. He pointed out that the eye symptom was readily relieved when cod-liver oil was administered, and called attention to the popular belief in the efficacy of chicken livers and eel fat as a remedy for this eye disease in children.

Falta and Noeggerath (1906), before the vitamin hypothesis was formulated, described a conjunctivitis with crust formation beginning about the fourth week and lasting until death in rats which were fed a diet which from their description must have been very deficient in vitamin A. Several other observers subsequently called attention to an edema of the eyelids and xerosis of the conjunctiva in animals fed a diet which we now know was lacking in vitamin A.

Stepp (1909) was the first to show by conclusive experiments that some nutrient principle not identifiable with any

of the known lipins was necessary for the maintenance of health in grown mice. Stepp's work was supplemented in 1912 by the author and by Fingerling (1912), who demonstrated independently that all the complex lipins which occur in the egg yolk or which are necessary for growth in birds can be synthesized from some non-lipin substances in the diet.

Up to 1912 all students of foods believed that fats had essentially the same nutrient qualities irrespective of their origin or nature. The demonstration in 1913 that certain fats had nutritive qualities not possessed by other fats equally digestible and palatable marked a milestone in the progress of nutrition studies, since it showed that by properly planned experiments on animals hitherto unsuspected qualities in foodstuffs could be demonstrated. McCollum and Davis (1913) were the first to plan an experiment which conclusively showed that butter fat and the fats of egg yolk contain something which is essential for the nutrition of the rat and which could not be provided by including lard or olive oil in the ration. Soon afterward Osborne and Mendel confirmed these observations on butter fat and lard, and added almond oil to the list of fats which were ineffective in inducing growth. They showed that cod-liver oil possesses the same properties as butter fat and egg yolk fat, and called attention to what they believed to be an infectious eye disease which was relieved by the administration of the "growth-promoting fats."

Bloch observed many cases of xerophthalmia in children in the vicinity of Copenhagen during the years 1912 and 1916. These children were fed a diet consisting of separator skimmed milk practically free from fat. The milk had been pasteurized

and cooked again in the home. Oatmeal and barley soup constituted the other important ingredients of the diet. Bloch referred the malnutrition in these infants to lack of fat in the diet, and observed that the eye trouble could be relieved by the administration of cod-liver oil, whole milk, or cream mixtures.

During a period of several years McCollum and Simmonds, who had repeatedly observed this peculiar form of ophthalmia in animals subjected to faulty diets, collected numerous observations on the experimental groups of rats in their colony where the ophthalmia occurred. A study of the data revealed the fact that many groups of rats in various degrees of enfeeblement or arrested development, the result of restriction to faulty diets of various kinds, failed to develop the disease, while others in adjacent cages were suffering from it. They found the ophthalmia to occur only in animals whose diets were deficient in the vitamin A, and in 1917 they correlated their observations on rats with those reported by Bloch and Mori, and expressed the view that the xerophthalmia or keratomalacia produced experimentally in animals is the analogue of the condition which these authors had reported in man. Since the publication of these observations the development of xerophthalmia has been generally accepted as the most characteristic sign of a lack of the vitamin A.

The view has not infrequently been expressed by clinicians that the ordinary mixed diet on which we live is satisfactory for the promotion of health, since the well-marked deficiency diseases occur only among people whose diets are notably restricted and monotonous. Evidence has been steadily

accumulating that this view is fallacious. As an illustration, Cramer (1924) describes epidemics of eye affections which occurred in England during the last forty years in various industrial schools. The last recorded outbreak was in 1911 and was investigated by McNeil and McGowan (1913). The condition of the children was diagnosed as "distorted pneumonia" which varied from a fulminating rapidly fatal type to an abortive or latent type. Special mention was made of the prevalence of a chronic granular conjunctivitis. Investigators clearly recognized the non-contagious nature of the epidemic, but were unable to explain the nature of the disease or to recommend a rational treatment. From our present knowledge of quality in foods it seems highly probable that these boys were manifesting the effects of vitamin A starvation. Several observers have correlated faulty dietary with the incidence of hemeralopia. Such relationship was described in 1883 by DeGouvea, who observed the disease among the negro slaves working on coffee plantations of San Paulo, Brazil. Little, Grenfell, Appleton, and others have mentioned the prevalence of night blindness in Newfoundland and Labrador. Blegvad (1923) studied the utilization of a preparation of vitamin A when injected subcutaneously. He estimated his preparation to have about 100 times the potency of cod-liver oil. He asserts that the utilization is much more rapid when this vitamin is introduced subcutaneously than when fed by mouth.

Osborne and Mendel (1917) observed calculi of calcium phosphate in the urinary tract in ninety-one animals among 857 necropsies. Forty-three per cent of these had not had a

satisfactory supply of vitamin A. In McCollum and Simmonds' experience calculi have occurred so frequently in animals whose diets contained an abundance of this factor, but were faulty in other respects, that it would seem to be the result of general debility rather than lowered vitality brought about by specific cause.

Wason (1921) studied the pathology of ophthalmia of dietary origin. She observed in the eyes of rats in which the disease was induced by selective fasting for vitamin A, hyalinization or necrosis of the outer layer of corneal epithelium, exudation of serum and cells into epithelium and stroma, and a proliferation of blood-vessels and fibroblasts. In advanced cases invariably the anterior and occasionally the posterior chambers were invaded. She concluded that the type and virulence of the organisms of secondary infection determine, in part at least, the course of the disease.

Yudkin and Lambert (1922) found that the changes in the eyes resulting from lack of vitamin A do not begin in the cornea, but have their origin in the lid. In this respect the sequence of events is the same as that of some of the severer types of acute and chronic conjunctivitis which are frequently complicated by corneal injury with infection and ulceration of this structure. They also say that the lacrimal gland may be the seat of marked pathologic changes either degenerative or inflammatory in nature. They observed variation in size and form and in staining properties of the cells, which they refer to functional disturbances related to the ophthalmia.

Mori (1922, 1923) studied histologically the eyes of rats in various stages of specific starvation for vitamin A. He

found the first observable change to be a tendency for the lacrimal gland to go into a resting stage, and to cease to produce tears. He believes that subsequent changes in the eyes can be accounted for as the result of loss of function of the tear glands. When the supply of tears fails the conjunctival sac is no longer washed continuously as under normal conditions, and bacteria quickly begin to grow there in great numbers. The growth of bacteria stimulates a migration of leukocytes, which accumulate in the eye chamber, causing the well-known hypopyon, which is visible as a yellowness in the pupil. Some of the leukocytes migrate through the outer coating of the eyeball and find their way into the conjunctival sac. Through their dissolution albuminous material accumulates which forms a sticky exudate that tends to paste the eyelids together. The drying of this exudate makes it difficult for the animals to get their eyelids open. It was found possible to cultivate ever-increasing numbers of bacteria from swabs taken from the surface of the eyeball day by day during the progressive fasting for vitamin A. Owing to dryness of the eye there is cornification of the external coating, the cells become flattened and pile up in pseudo-stratified form, resembling horny layers of skin. Ulcers regularly form on the cornea during the later stages of the disease owing to the death of the tissue. The ulcers finally perforate and the lens pops out. It is possible to induce spectacular recovery in animals whose eyes are in a very damaged condition by administering fats containing an abundance of vitamin A. Mori regards the ophthalmia of dietary origin as an analogue in certain respects at least of

a human disease common in many parts of the Orient and known as hikan.

Sections of the lids of the eyes of animals suffering from xerophthalmia very often show cystic dilatation of the ducts of the meibomian glands. These cysts are filled with fat, the secretion of the gland cells. The epithelium of the margin of the lid shows evidence of a very marked xerotic process, and the same change is found in the epithelium of the duct. It is very likely that this xerosis may play a part in the occlusion of the duct and the formation of the retention cysts.

The lumina of the acini of the harderian gland may be either very much dilated and empty, or narrowed by swelling of the secreting cells. Frozen sections of this gland show a remarkable diminution of the fat content of the cells and the lumen. The connective tissue about the gland acini is very often densely infiltrated with round cells.

The mucous cells of the conjunctiva are all entirely destroyed in the course of the xerotic process. It is certain from these findings that the entire secretory apparatus of the eyes of these rats is in a state of dysfunction. The secretion from these glands is either very much diminished or entirely lacking. The changes in the lacrimal gland are the most important in the pathologic picture of this disease, and, in fact, the changes in this gland would seem to be the cause of the lesions in the cornea and conjunctiva. The diminution or the lack of the secretion of the lacrimal gland would explain the dryness of the eyeball as well as the xerosis of the cornea and the conjunctiva. The failure of the lacrimal secretion

also explains the increase in number of the organisms found in the conjunctival sac.

Mori also examined the salivary glands of rats suffering from lack of vitamin A, since both in nerve supply and in structure these resemble closely the lacrimal gland. The submaxillary and parotid glands of the rat are serous; the sublingual is a mucous gland. In many rats with xerophthalmia either all or some of these glands are either not secreting at all or secreting very little. The secreting cells become much shrunken, the acini are very small and show no traces of secretion. The epithelium of the intralobular ducts is shrunken and the cells are irregular in size.

The epithelium of the principal ducts of these glands often shows cornification and desquamation of the thickened superficial cells, so that the lumen of the duct becomes narrow and is often occluded. Dilatation of the small ducts occurs frequently in the parotid gland. Coincident with the xerotic changes and those of the parenchymal cells, the ducts are invaded by bacteria, and small abscesses are formed in the gland.

The other secretory organs, such as the liver, pancreas, bowel, and kidneys, showed no remarkable change except in one case. In one animal the cytoplasm of a small number of cells of the pancreas was very much vacuolated. The reproductive glands of the rat on a diet deficient in vitamin A do not function.

Evans and Bishop (1922) found a characteristic disturbance of the œstrual cycle in the rat caused by a deficiency of vitamin A. This resembled no other nutritional upset known.

It consisted of a prolongation of the œstrual desquamative change in the vaginal epithelium. The smear consisted chiefly, if not exclusively, of cornified cells which in the normal individual characterize the actual period of œstrus and ovulation only. In the case of animals showing a deficiency of vitamin A these cells occur during the entire period of the acute deficiency. This, they state, may constitute the only sign of deficiency of vitamin A except the failure to reproduce successfully.

Cramer (1923) studied the pathologic lesions induced by deficiency of vitamin A and interprets his observations as pointing to the digestive tract as the key to the problem of the mode of action of this accessory substance. He believes the functional integrity of the digestive tract to be dependent on the presence in the food of certain vitamins. He believes these have a specific drug-like action analogous to the effect on the functional *inactivity* of the uterus by a hormone produced in the ovary. The vitamin A has, according to his view, a specific stimulating effect on the intestinal mucous membrane, and also directly or indirectly on the formation of blood platelets. Cramer's histologic studies on sections of the rat's intestine, made "upward from the cecum," indicate that on a vitamin-free diet, about the time xerophthalmia begins to develop, there is seen a profound atrophy of the villi and necrosis of the upper parts of them. Rats in an advanced stage of deficiency of vitamin B, but receiving an abundance of vitamin A in cod-liver oil, had villi which deviated in appearance from the normal, but showed no atrophy and no necrosis. Cramer asserts that the vitamins have a

positive action, and he believes he has demonstrated an important stimulating effect on the process of food absorption from the intestine in perfectly normal animals, which have never been subjected to any dietetic deficiency, when the vitamin moiety in their diet is markedly increased.

Besides these gross changes an abundance of protozoa, mainly *Giardia intestinalis*, were found in the lumen of the intestine of rats in advanced deficiency of vitamin A. These are presumably present in small numbers in the intestine, but the absence of vitamin A enables them to proliferate rapidly and to penetrate between the villi where normally they are never present. This fact, Cramer points out, may be of importance in determining the pathogenicity of certain organisms. They may be non-pathogenic on one diet and become pathogenic by variation of the pabulum in the intestine.

The intestinal bacteria also appear to increase greatly in number over what is normally seen, and, what is more significant, may often be seen adherent to the villi, especially the necrotic tips. Normally the bacteria remain confined to the center of the lumen. In deficiency of vitamin A Cramer found the bacteria often invaded the mucous glands and were found in dense masses filling the lumen of the gland to the bottom.

Mention has already been made of the view of Cramer that, among other effects, the vitamin A produces directly or indirectly a stimulation of the formation of blood platelets. Cramer, Drew, and Mottram (1922) reported that a diet deficient in vitamin A produces a progressive decrease

in the number of blood platelets, and that thrombopenia was the only constant lesion for vitamin A deficiency and was characteristic of this deficiency just as lymphopenia was characteristic of vitamin B deficiency. Thrombopenia may be present in rats which are on a diet free from vitamin A before they show obvious signs of ill health. When a profound thrombopenia had been established the addition of vitamin A to the diet produced a rapid increase in the platelets to the normal number. Exposure to radium produced a lymphopenia, and also a thrombopenia if the dose was sufficiently large, but the animals recovered rapidly if the radium treatment was discontinued and the dose had not been too large.

The views of Cramer and his co-workers have been contraverted by Bedson and Zilva (1923), who report that rats fed diets deficient in vitamin A show a decrease in the number of platelets, but believe the decrease too small to be significant. Cramer, Drew, and Mottram maintain, however, that the technic of Bedson and Zilva was not sufficiently refined to justify confidence in their findings, to which Bedson and Zilva reply that on repeating the work with the same technic described by Cramer and his co-workers they were still unable to confirm their observations. The relationship of deficiency of vitamin A to blood-platelet formation, therefore, cannot be looked on as established.

Origin.—It is certain that vitamin A is synthesized by flowering plants, for it is present in the leaves of all plants thus far examined. The source of vitamin A in fish oils has been definitely traced by Coward and Drummond to marine

algæ containing chlorophyl. Other lower marine plants differently adapted to photosynthesis (red weeds) are not so active in producing the vitamin, while those devoid of pigment which play a rôle in carbon assimilation (mushroom) are almost completely deficient in it.

Occurrence of Vitamin A.—Vitamin A is found most abundantly in certain foods of animal origin. Cod-liver oil appears to be the richest of all fats in this substance, but fish oils in general are good sources of it, as are also egg yolk fats and butter fat, the lipin extracts of glandular structures, such as liver, kidney, and testis. Vegetable oils, without exception, have proved to be either deficient in vitamin A or to contain but small amounts of it. Among the vegetable products, leaves of plants, such as spinach, alfalfa, and celery leaves are rich in vitamin A, whereas those leaves which are thickened storage organs, such as cabbage and cauliflower, are more comparable to the tubers in that their content of vitamin A is low. An interesting correlation was proposed some years ago by Steenbock between yellow pigmentation in plants and vitamin A content. He demonstrated in the case of several products a striking relationship between yellow pigmentation and vitamin A content. Thus, he found white corn to be deficient, whereas yellow corn was moderately rich in vitamin A. Yellow turnips and sweet potatoes contain vitamin A, whereas colorless varieties do not. Red and blue corn did not contain the vitamin A unless yellow pigmentation was also present. Red pigmented vegetables, such as the tomato, contain much yellow pigment masked by the red, and are correspondingly rich in the vitamin.

Palmer and Kennedy have shown clearly, however, that the relationship between vitamin content and yellow pigmentation is a chance one. Pig liver is free from the carotin and xanthophyll pigments so widespread in the plant world, but is rich in vitamin A. The fats in the milk of certain species of animals, such as sheep, goat, swine, and rat, may be as white as lard, yet have a high content of vitamin A. Whereas the glandular organs of animals are rich in vitamin A, the muscle tissue is a very poor source of it. The body fats of animals may contain considerable vitamin A provided the food has been rich in this substance, or practically devoid of it if the vitamin was not provided in the ration. A number of investigators have shown clearly that the vitamin A is not present in milk fat unless it is supplied in the dietary.

Properties.—Miss Davis and I (1914) observed that the vitamin A was still present in yolks of hard-boiled eggs, which demonstrated a moderate degree of stability toward heat. We also demonstrated that butter fat could be saponified without destroying its vitamin A content. Osborne and Mendel passed live steam through butter fat for two and a half hours without producing marked deterioration in vitamin value. Steenbock, Boutwell, and Kent (1918) found that heating butter for four hours at 100° F. caused the destruction of its vitamin A content. The reason for this was first pointed out by Hopkins, who showed that oxidation is a factor of prime importance in determining the rate of destruction of the vitamin during the heating of foods. The vitamin A, therefore, is stable at high temperatures in the absence of oxygen.

Vitamin A is not extracted to an appreciable extent from plant products when the fats are removed by such solvents as ether, chloroform, benzene, or acetone. Hot alcohol is a much better solvent for its separation. Osborne and Mendel (1918, 1919) have shown, however, that ether or benzene extracts a certain amount of vitamin A from leaf structures, whereas water does not remove it. Emmett and Luros (1919) showed that benzene or acetone will not extract vitamin A from pancreas, thymus, and adrenals. Steenbock and Boutwell (1920) found that when carrots are saturated with lard or corn oil and then extracted with ether, little or none of the vitamin A is removed. Chloroform and carbon disulphid remove considerable amounts of vitamin A from carrots.

Takahashi (1924, personal communication), in the laboratory of U. Suzuki, reports the isolation of vitamin A from cod-liver oil, butter fat, and green laver. He calls the substance biostearin. It has the formula $C_{22}H_{44}O_2$. It is believed to contain two hydroxyl groups, one of which is tertiary, and the other primary or secondary. It is, therefore, an alcohol closely related to cholesterol. It is believed that cholesterol is a product of biostearin after it has undergone a physiologic change in the body. When 0.0001 per cent of biostearin is present in the diet of the rat, health and growth are maintained. He states that the effect of biostearin is proportional to its concentration in the diet up to 0.05 per cent, but when this amount is exceeded it has an unfavorable effect.

Takahashi has prepared the benzoate, acetate, hexabromid, and ozonid of the substance. It has a molecular weight of about 400.

When a small amount of biostearin was placed in a dish and covered with a dry plate the image of a screen placed between the plate and the biostearin appeared on the plate. This was true even when the plate was at a considerable distance from the biostearin. This happened even though the plate was carefully wrapped in black paper of the kind used to protect dry plates in commerce. When biostearin was sealed in a quartz tube and taken into a dark room, an image of an object between them was produced on a dry plate kept near the tube. The action of biostearin on the dry plate was much less when glass intervened than when quartz was used.

When biostearin was placed in a flask and a current of carbon dioxid was passed over it and the gas was led out through an outlet before which a dry plate was held so that the gas which had been in contact with the biostearin was brought into contact with the plate no action was observed. When a dilute solution of biostearin was spread upon a dry plate and immediately washed off, the plate was not affected. When a dry plate was partly immersed in an alcoholic solution of biostearin the submerged part was not affected, but the part of the plate outside of the solution was affected. Reduced copper or aluminum foil kept in the same vessel with biostearin was rapidly oxidized. In the environment of biostearin something is formed in the air which gives the KI-starch reaction and the paranitrophenylenediamin reaction. It is believed that it in some manner activates oxygen. Biostearin is a colored substance resembling the carotinoids, but unlike these it does not have a selective absorptive action

in the visible part of the spectrum. An alcoholic solution of the substance gave an absorption band near 3,200 A of the ultraviolet region. The solution becomes fluorescent when illuminated with light from the iron arc.

Requirements of Different Species for Vitamin A.—Deficiency of vitamin A has produced ophthalmia in man, guinea-pig, rabbit, dog, swine, and chicken. Sugiura and Benedict (1922) drew the conclusion from experiments on pigeons that vitamin A is not essential in any stage of avian nutrition. They also found pigeons to remain in health on a diet lacking in vitamin C.

Emmett and Peacock (1923, 1924) found that young chickens require vitamin A, but that young pigeons do not suffer from deprivation of this substance. It appears, therefore, that the pigeon deviates from the general rule among birds in that either it does not require the vitamin A or is able to synthesize this substance. Beach (1923) described a pathologic condition in chickens caused by lack of vitamin A which would ordinarily be diagnosed as avian diphtheria or roup. The disease was shown not to be infectious and could be controlled by giving foods rich in vitamin A. He described the development of xerophthalmia in chickens, and also found a remarkable accumulation of crystals of urates in the kidneys of chickens deprived of vitamin A. The kidneys were also pale and marked with a network of fine lines which are urate-filled tubules. White crystalline material, probably urates, was frequently noted on the liver and other organs.

VITAMIN B

American and English investigators are apparently convinced that beri-beri is essentially a disease due to lack of vitamin B. In conversation with several eminent Japanese physicians I have found them still unwilling to admit that this is the sole cause of the disease.

Walshe (1918) asserted that there are two factors in the production of beri-beri, the absence of a vitamin and the use of certain foods which are the direct and immediate cause of the disease. He suggests that in the absence of a specific vitamin carbohydrates undergo an aberrant hydrolysis with the production of toxic by-products, thus producing beri-beri. According to his view it is essentially an intoxication. Vedder (1923) suggests that beri-beri may be caused by the lack of two vitamins. In this way he would account for the two forms known as wet and dry beri-beri. McCarrison (1924) likewise insists that beri-beri is not directly a disease due to deficiency of vitamin B. He believes there are endemic areas in the Madras Presidency where people suffer from beri-beri on dietaries which would protect them in non-endemic areas. There can no longer be any question that experimental beri-beri or polyneuritis as produced in mammals in the laboratory is due to deficiency of vitamin B.

Vitamin B is the most widely distributed of any of the known vitamins. It is present in all natural foodstuffs. Only manufactured products, as polished rice, white wheat flour, degerminated cornmeal, corn grits, and sugars, are essentially lacking in it. The vitamin B is more stable than either vitamin A or C. It appears to be an organic base and is

essentially insoluble in all solvents other than water, aqueous alcohol, and glacial acetic acid. Its isolation is attended with special difficulty because it occurs only in complex foodstuffs, and extracts which contain it are likewise contaminated with very large amounts of numerous impurities. The substance occurs in but very small amounts in any foods. Yeast and the germ of wheat are two of the foods richest in this substance.

Studies on Isolation of Vitamin B.—Williams (1917) expressed the view that under certain conditions alpha-hydroxypyridin may possess antineuritic properties. He attributed these properties to the existence of this compound in the form of a pseudo-betain, and suggested that a configuration confirming more or less closely to that of the betain ring was probably an essential characteristic of the vitamin B. It was pointed out that such a structure was possible in most of the simpler nitrogenous components of animal tissues, especially in the purin bases. Williams had previously brought forward the view that adenin may exist in a labile form in which it possesses curative properties for polyneuritic pigeons. Dutcher (1919) asserts that desiccated thyroid, thyroxin, pilocarpin hydrochlorid, and other physiologic stimulants are able to induce "cures" with pigeons in polyneuritis.

Besides alpha-hydroxypyridin, Williams has also stated that alpha-methylpyridone, trimethyl uracel, and 4-phenyl-iso-cytocine gave slight protection. He considers vitamin B to be a cyclic nitrogen compound with a substitution of oxygen in the ring and capable of existing in the betain configuration. Funk reported cures in the pigeon with hydan-

toin, and observed some curative properties in adenin and pyrimidin derivatives.

Osborne and Wakeman (1919) described a method for preparing an extremely potent preparation of vitamin B. They prevented disintegration of yeast cells by boiling in acidified water after thorough washing. The protein was then coagulated and an extract, free from the products of autolysis, was obtained. The extracts from 4.5 kilos of fresh yeast, extracted with 15 kilos of water, were concentrated to 2 liters and poured into 3 liters of 93 per cent alcohol. The precipitate which formed was inactive. The filtrate was evaporated and again poured into alcohol and the process was again repeated, so that a precipitate was finally obtained in an alcohol of 90 per cent strength. This amounted to 6.2 per cent of the dry yeast and contained almost all of the vitamin B in the yeast. This preparation was not composed of pure vitamin, although it was extraordinarily active biologically.

Shinza (1924) states that the symptoms of experimental polyneuritis are strongly like those due to depletion of the potassium in the body, and that the administration of potassium salts to polyneuritic birds had a restorative effect.

Levene and van der Hoeven (1924) have prepared a more potent preparation of vitamin B than that secured by Osborne and Wakeman. For the preliminary concentration of the material they employed the method of Osborne and Wakeman, then adsorbed the vitamin on silica gel. From this it can be extracted by alkalies and also by acids of pH_3. They found that barium hydroxid incompletely precipitates

the vitamin from its solution, but a preparation was secured which they estimated to be 200 to 400 times as potent as that of Osborne and Wakeman. This material is obtained with very slight loss of vitamin. The preparation of Levene and his co-workers was active in daily doses containing 0.00017 gm. of nitrogen per day.

It was pointed out by McCollum and Simmonds (1918) that the pigeon test is uncertain, and that there is a high degree of improbability that so many totally unrelated chemical substances, as pyrimidins, purins, and pyridin, could fulfil the same physiologic purpose. They were led to suspect that the curative substances are not necessarily identical with the indispensable nutritive principle essential for growth and normal functioning, but rather bodies which possess the pharmacologic properties of stimulating certain nerve cells to renewed activity. McCollum and Simmonds described a procedure for determining the presence or absence of the vitamin B. It can also be made roughly quantitative. The procedure is to restrict young rats to a diet of purified protein, dextrin, a salt mixture, and butter fat, or other fat which contains vitamin A. On such a diet the animals are unable to grow and ultimately die with or without the development of symptoms of polyneuritis. After from three to five weeks, while the animals are declining in weight, the substance to be tested is administered. If the animals recover and resume growth the test is positive. If they continue to decline, the substance tested for is absent or present in amounts too small to meet the nutritive needs. This test requires considerable amounts of material. Only after

a week or two is the outcome apparent. Levene has adopted a modification of the growth test with the rat. In this test he employs rats about six weeks old and uses our basal diet for the production of polyneuritis. The animals are kept on this for three weeks. The average weight of his animals was 50 gm., and during the week immediately preceding the test they lost on the average 5 to 10 gm. The substance to be tested is given in solution. After from three to four days the animals are again weighed and the change in weight is accepted as an indication of the vitamin content of the solution.

Seidell (1924) has prepared very potent preparations of the vitamin B by taking advantage of the fact that Fuller's earth exerts a selective adsorption for this substance as well as for alkaloids. From this "activated" Fuller's earth the vitamin can be separated by treatment with barium hydroxid. From such concentrated preparation Seidell has prepared a silver compound and also a picrate of extraordinary potency when tested on pigeons. This picrate, although of extraordinary potency, is not a pure compound, since the crystalline product, of which 2 mg. daily protected pigeons, was not composed of homogeneous crystals, but of crystals of more than one kind.

There is no convincing evidence that we know anything about the chemical nature of the vitamin B. We are not entirely certain that it is a single substance, and not several substances acting together. It is stable at high temperatures in acid solution, but in alkaline solution rapidly becomes denaturized. It is not destroyed by nitrous oxid, and so

appears not to be either a primary or secondary amin. McCollum and Simmonds have tested a number of the substances which are said by others to induce a cure of experimental polyneuritis in pigeons with the growth test in rats, and have invariably found that none of these exert the slightest beneficial effect on the polyneuritic rat. For this reason they have expressed skepticism concerning the specificity of the pigeon test for the vitamin B. This raises the question as to whether or not the nutrient requirements of the pigeon and the rat with respect to this substance are identical, a question which still remains to be decided.

Relation of Histamin to Vitamin B.—Voegtlin and Myers (1919) called attention to the fact that vitamin B reacted to chemical reagents such as methyl alcohol, silver, lead, and barium salts in a similar way to secretin. Secretin preparations from the duodenum of dogs relieved, to some extent, the antineuritic symptoms, and the vitamin B from brewer's yeast on injection into dogs stimulated the pancreatic and biliary secretions. A yeast preparation which had lost its curative powers for avian polyneuritis was devoid of any stimulating effect on the pancreatic secretion and bile flow. Anrep and Drummond (1921) found that a yeast extract does not cause secretion of pancreatic juice as does secretin. Secretin can be extracted from the intestine of a cat showing polyneuritis. The suggestion of Voegtlin and Meyers that vitamin B and secretin are identical is not supported.

Cowgill (1921) reports that intravenous injection of extracts of rice polishings, of wheat embryo, of navy beans, of yeast, or of neutralized tomato juice, which all contain vitamin B,

does not stimulate the flow of saliva as does pilocarpin. Similar extracts were found by Cowgill and Mendel (1921) to be without noticeable effect on the rate of flow of pancreatic juice, bile, or saliva in the dog. The intestinal mucosa of polyneuritic dogs was shown to contain secretin. No direct relationship has been established, therefore, between vitamin B and the secretory functions of the pancreas, liver, or salivary glands.

Boyenval (1922) stated that on injecting histamin into rats fed polished rice he found no effects from the cachectic progress of the disorder. On the other hand, the histamin-treated rats did not show the usual premortal nervous disturbances observed in the control animals. He thought histamin may exercise an antineuritic effect. Koskowski (1922) confirmed the findings of Boyenval. Although histamin stimulates the activity of the digestive glands, especially of the gastric glands, and thus aids in the rat's digestion, it does not supply the lack of nitrogenous substances found in the pericarp of the rice grains and elsewhere, and it cannot, therefore, replace the antineuritic vitamin. Burge (1916, 1917), Dutcher (1920), and Stehle (1919) have presented conflicting observations concerning the body content of catalase in polyneuritis and its possible rôle in the pathology of that disease. Nothing definite has been established. Findlay (1921) reported a study of the effect of deprivation of pigeons of the vitamin B on the content of glyoxalase in their tissues. Glyoxalase is an enzyme which plays a rôle in the metabolism of carbohydrate. It has the power to transform "glyoxals" into lactic acid. The "glyoxal" which serves in the animal body

as the intermediate substance between glucose and lactic acid is known as pyruvic aldehyde. Findlay found the amount of glyoxalase in the livers was reduced about one-half in polyneuritis. On inducing a "cure" by the administration of the vitamin B the glyoxalase rose rapidly to two-thirds of the normal amount.

Nature of Action of Vitamin B.—Green (1918) states that the daily requirement of pigeons for vitamin B varies with the exogenous metabolism. He believes there is no more reason to think that vitamin consumption is related to carbohydrate metabolism than it is to protein or fat. Funk (1919) tried the effect of various substances which were known to influence carbohydrate metabolism, on the body sugar, liver glycogen, onset of beri-beri, loss of weight and length of life in normal pigeons and in others fed polished rice exclusively. The substances tried were dextrose, phloridzin, adrenalin, thyroid, parathyroid, and pituitrin. He concluded that the antiberi-beri vitamin played an important rôle in carbohydrate metabolism. Vedder has, however, given experimental evidence which is opposed to this view.

Dutcher (1918) was of the opinion that the carbohydrate effect was due to an overloading of the oxidative mechanism of the body rather than to a specific relation between the metabolism of carbohydrate and vitamins, the latter being used up in the process. He believes that in specific fasting for vitamin B there is an accumulation of incompletely metabolized products which affects the nervous system and accounts for its loss of function. He further stated (1920) that the antineuritic substance functions as a metabolic

stimulant, since the body temperature fell during the development of avian polyneuritis and rose after giving vitamin B. He observed the catalase content of the tissues of birds suffering from the disease to be decreased to 56 per cent of the normal. It returns to normal when vitamin B is administered. He interpreted his results as indicating a reduction of the oxidation processes in polyneuritis. Such a depression of oxidation results, he believed, in the accumulation of toxic metabolism products which affect the nervous system.

Karr (1920) studied the effect of specific fasting for the vitamin B on the desire of dogs to take food. He fed diets composed of isolated food substances and especially free from vitamins A, B, and C. The dogs were restricted to this diet until they refused to take the food, and then a source of vitamin B was fed separately. Yeast, milk, tomato, and a concentrated extract of vitamin B were employed for this purpose. The addition of these substances resulted in prompt response of the appetite. Karr concluded that there was some relation between the desire for food and the amount of vitamin B ingested. Brewer's yeast was much more effective than baker's yeast for this purpose. He also studied the metabolism of dogs deprived of vitamin B by making quantitative studies of the assimilation of nitrogen. No decrease in the capacity for digestion nor in the character of the metabolic products eliminated could be detected as the result of specific starvation for this substance. He found that vitamin A had no such effect in influencing the appetite as did vitamin B. Cowgill (1921) confirms Karr's view that

"there is some relationship in dogs between the desire to partake of food and the amount of so-called water-soluble vitamin ingested." Kennedy and Dutcher (1922), on the other hand, insist that the effect of vitamins is not necessarily one of body stimulation, but rather a stimulation of the metabolic processes which promote growth or normal functioning. Theirs appears to be the more logical view.

Pathology of Beri-beri.—A new conception of the pathology of polyneuritis and other deficiency diseases was introduced by McCarrison (1919). Loss of the co-ordinating powers of the muscles is the most striking feature of polyneuritis. The onset of the disease is generally preceded by the bird sitting with ruffled feathers and with the appearance of illness. There is progressive weakness, and when disturbed there is a tendency for many pigeons to be taken with convulsive seizures in which they turn "cart-wheels" backwards at intervals. In the acute type of the disease many birds sit with the head greatly retracted. These symptoms generally led investigators to accept the view that the lesions in beri-beri were principally situated in the nerve system. McCarrison has presented evidence that injury to the nerves is much less pronounced than injury to certain other tissues. He observed functional and degenerative changes in the thymus, testicles, spleen, ovary, pancreas, heart, liver, kidneys, stomach, thyroid and brain, atrophy being apparent in every case, the severity being in the order named. The adrenals, on the other hand, suffered hypertrophy. This was associated with a proportionate increase in the content of the glands in adrenalin. Edema was invariably associated with

hypertrophy of the adrenal glands, suggesting its relation to an excessive production of adrenalin. Inanition gave rise to adrenal hypertrophy and atrophy of other organs, the brain excepted, similar to that observed in birds fed solely on polished rice. Rice is deficient in all known vitamins, protein, and in several inorganic elements. The gastric, intestinal, biliary, and pancreatic disorders of birds fed only polished rice were more serious than the nerve lesions. This diet gave rise to congestive and atrophic changes in all the coats of the intestine, especially the duodenum; to lesions in its neuromuscular mechanism; to impairment of its digestive and assimilative functions; and to failure of its protective resources against bacterial infection. Guinea-pigs restricted to a diet of oats and autoclaved milk developed lesions of the digestive tract analogous to those seen in pigeons fed polished rice. Owing to the multiple deficiencies of the rice diet it is not possible to decide as to the specific effects of deficiency or lack of a single dietary component. It is interesting to note, however, that on a diet deficient only in vitamin C, guinea-pigs developed lesions comparable with those in birds with diets faulty in several respects.

McCarrison fed pigeons a diet of polished rice, butter fat, and raw onions. Typical polyneuritis developed. In these birds atrophy of the myenteron and of the elements of the mucosa were often comparatively slight. The results of this experiment are very significant for the interpretation of much of McCarrison's data, for with a diet of polished rice, butter fat, and onions the most pronounced deficiency was in the antineuritic principle. Certain inorganic elements,

especially calcium, phosphorus, and potassium, were supplied in very inadequate amounts, and the intestinal lesions produced were less pronounced than with a diet of polished rice alone. The addition of vitamin A in butter fat and of vitamin C in onions apparently improved the well-being of the birds to a marked degree. The confusion in this field is well illustrated by mentioning again in this connection that certain investigators believe that the pigeon requires neither vitamin A nor C.

In a number of McCarrison's polyneuritic birds the blood was found to be infected by *Bacillus suipestifer*, *Bacillus pyocyaneus*, and another organism not identified. He emphasized his belief that systemic infection is rendered easier by the presence of the pathologic processes existing in the intestine as the result of dietary deficiencies. Such invasion was favored by the impaired production of digestive juices by the malnutrition of the secretory cells owing to the continued congestion of the mucous membrane of the intestine and the blood, by the greater opportunity which the debilitated mucous membrane provided for the growth of micro-organisms on its surface, and to actual breaches of continuity in the walls of the bowel itself. The imperfect digestion in the upper part of the tract offered a favorable medium for the growth of bacteria. Unwholesome products, he believes, tend further to debilitate the mucosa and to increase the prospects of invasion by micro-organisms. Infection of the blood from the bowel under such conditions would be expected and was repeatedly demonstrated by aërobic culture of the heart blood. It must be emphasized

in this connection that in no instance did McCarrison feed a diet complete except for a lack of the vitamin B, and so it is not yet possible to determine which of the pathologic effects that he observed were the result of vitamin B deficiency and which were caused by other deficiencies in his experimental diet.

Cramer, Drew, and Mottram (1921) observed that the absence of vitamin B from the diet of mice and of rats led to atrophy of lymphoid tissue throughout the body, and also to lymphopenia in the circulating blood. The number of polymorphonuclear leukocytes was not affected. The absence of vitamin A did not lead to atrophy of the lymphoid tissue and there was no lymphopenia. They found that absence of the vitamin B led to characteristic nutritional disturbance, such as loss of weight, emaciation, subnormal temperature which may be designated by the term "marasmus." No such marasmic condition resulted from a lack of vitamin A. This condition can be produced by other processes which destroy lymphocytes, such as *x*-ray exposure. They conclude that vitamin B is necessary for the normal functioning of the lymphoid tissue. These investigators (1922) made a histochemical study of fat absorption from the intestine. They demonstrated that the functional activity of the intestinal epithelium as regards absorption is profoundly affected by the presence in the food of vitamins A and B, particularly the latter. They believe that the vitamins have a stimulating action on absorption and probably also on intestinal digestion. In the absence of vitamins they found no delay in the passage of food in such animals. After exposure

to radium sufficient to produce lymphopenia the absorption of food was impaired in the same way as if vitamins A and B were absent, and this effect could not be counteracted by an abundant supply of vitamins in the food. These observations confirm the view previously expressed by the authors that vitamins play an important part in the absorption of food, and the marasmus resulting from a deficiency of vitamin B is due to an impaired assimilation from the intestine.

Findlay (1923) observed that diets lacking in vitamin B produce in pigeons and rats definite changes in the hematopoietic tissues, consisting of congestion and hemorrhage in the bone-marrow, followed in the more chronic cases by gelatinous degeneration. He noted that pigeons fed a diet lacking in vitamin B were much more susceptible to infection with pneumococcus and meningococcus, organisms to which they are naturally quite immune. They were also much more susceptible to *Bacillus coli* and *Bacillus enteritidis*. The reduction of natural immunity he found to be related to a fall in body temperature produced by diets deficient in vitamin B. It was only marked when the cloacal temperature was lowered to 40° C. or below. He stated that the lowering of the body temperature appeared to decrease the resistance by (*a*) facilitating the growth of the invading organisms, (*b*) reducing the leukocytic response to the infection, and (*c*) reducing the bactericidal power of the leukocytic exudate.

Very few investigators, even among biochemists, who are best in a position to understand the interpretation of quality in diet, are yet able to plan and prepare diets which are complete in every respect except for a single missing factor.

Bacteriologists, immunologists, and others are in many cases wholly unprepared to manage the dietary phase of their studies designed to show the pathologic effects of diets faulty in specific ways. The result is that much of the literature deals with experiments in which multiple defects were characteristic of the dietaries, and so interpretation is not possible. That very important discoveries are still to be made in the pathology of malnutrition there can be no doubt, but much more work and better work is necessary before these discoveries can be made. A recent observation by Webster and Pritchard (1924) is of special interest. They report that white mice from the Rockefeller Institute breeding room fed on the McCollum-Simmonds diet, consisting of whole wheat 67.5, casein 15, milk powder 10, sodium chlorid 1.0, calcium carbonate 1.5, and butter fat 5.0 per cent, are more resistant to typhoid infection, mercury bichlorid intoxication, and botulinus toxin than are similar mice fed on bread and pasteurized milk supplemented by an oatmeal and buckwheat mixture and dog biscuit. This comprehensive study emphasizes the necessity of feeding diet exactly controlled in every detail if one would secure optimal as contrasted with good nutrition; and it indicates that the body profits in a physiologic sense by an optimally constituted diet in the same degree that success is achieved in chemical operations following the best procedure.

BIOS AND THE GROWTH OF YEAST

Investigators have been very desirous of isolating and identifying vitamin B. We have as yet no qualitative tests which

characterize it, and accordingly any effort at separating this interesting substance must be controlled by feeding tests on every fraction or solution which appears of interest. Such a method is time consuming and requires an extravagant expenditure of material. As a result of this Williams sought to use yeast as a test organism for determining the content of vitamin B in extracts of natural foods. It was observed by Wildiers (1901) that yeast grew very slowly in a medium of pure sugar and inorganic salts where ammonium sulphate was the only source of nitrogen, unless the seeding was large. Pasteur had already observed that single yeast cells did not proliferate in a medium of relatively pure substances as did seedings containing many cells. Wildiers sought to explain this phenomenon by postulating the existence of a special nutrient principle necessary for yeast proliferation to which he gave the name "bios." Williams confirmed Wildiers' findings that extracts of certain substances, such as yeast cells, greatly stimulate the rate of proliferation of yeast. Williams was impressed by the fact that those substances which are richest in vitamin B gave extracts which were most potent in stimulating yeast growth, and conceived the idea that the stimulating substance was the vitamin B. Williams devised a procedure by means of which the stimulating effects of various extracts and various amounts of these on the proliferation of yeast cells during a period of eighteen hours could be readily determined by counting the progeny of a single cell produced during this interval.

Souza and McCollum sought to apply the method of Williams and discovered that the test was not a test for the vita-

min B, but for some other substance which affects yeast. Extracts of beef muscle or of rolled oats stimulate the growth of yeast in a purified medium, but the same is true of these substances made after alkalinizing the preparation and heating to a point where growth tests on rats indicate that the vitamin B has been destroyed. Obviously bios has a stability in the presence of alkali not possessed by vitamin B.

Nelson, Fulmer, and Cessna (1921) demonstrated that yeast growing in a purified nutrient solution at a slow rate synthesizes vitamin B. This was confirmed by MacDonald.

Utilizing this slow growth of yeast, MacDonald accumulated 6 to 14 gm. of dry yeast cells from several strains or pure organisms, transplanted at weekly intervals to fresh nutrient medium free from either bios or vitamin B. Such yeast when fed to rats declining in weight for lack of vitamin B led to a response entirely comparable to what one sees in such experiments when similar amounts of commercial yeast are added.

Fulmer and Nelson used a synthetic sugar, methose, prepared from formaldehyd, as the sole source of energy in a medium in which yeast was grown, and in the absence of both bios and vitamin B. Under these circumstances yeast can undergo cell proliferation which is greatly stimulated by the addition of the substance bios. The work of several investigators has been adversely criticized because of the assumption that the cane sugar employed in the medium still contained traces of vitamin B (bios).

Numerous studies have been made during the last four years on the effect of various preparations of yeast prolifera-

tion with a view to throwing some light upon the nature of bios. Fulmer and co-workers (1924) believe they have demonstrated that bios is not a single substance, since the combination of two extracts, neither alone very active, was much more effective.

Eddy, Kerr, and Williams (1924) have isolated from yeast a crystalline substance melting sharply at 223° C. They believe this substance to be a bios. The recrystallized product retains its melting-point and shows no loss of activity. The yield of the product was about 0.03 per cent of the dry yeast. It contains 43.29 per cent of carbon and 8.31 per cent of hydrogen. The substance gives no ninhydrin or biuret reaction. This substance, they say, stimulates the growth of yeast. Their crystalline preparations show but a single crystal form under the microscope. They assign the provisional formula $C_5H_{11}NO_3$, admitting possible variation in the hydrogen value. Notwithstanding this seeming conclusive evidence of the individual nature of the substance isolated, and its stimulating effect on yeast, Eddy and co-workers state: "Nothing in our method of isolation negates the possibility of more than one bios. In fact, the relative stimulation producible by the addition of our pure product and by the use of autolyzed (yeast) lends strong probability to the suggestion that the latter contains more than one growth stimulant." Miller (1924) and later Deas (1924) have presented extensive experiments strongly suggesting that there are at least two bioses.

VITAMIN C

There appears to be unanimity of opinion among all investigators that scurvy is a condition resulting specifically from a lack of the vitamin C. Modern knowledge of the etiology of scurvy dates from a discovery by Holst and Fröhlich (1912) that guinea-pigs rapidly develop the disease when confined to a diet of cereals or of bread. They observed that excessive feeding of guinea-pigs with carrots, turnips, or dandelions did not lead to the development of scurvy, although the animals on this food suffered considerable loss in weight. The addition of small amounts of fresh cabbage or carrots or other fresh vegetables cured animals suffering from scurvy. They showed that the antiscorbutic substance is destroyed by cooking or drying. It was definitely shown by Holst and Fröhlich (1922) that the antiscorbutic potency of fruits and vegetables displayed a greater stability in acid than in alkaline solution. They observed that fruit juices were more heat stable than vegetable juices, and suggested that the acidity protected the antiscorbutic principle. Fürst (1912) found that whereas dry cereals or pulse did not prevent scurvy, they acquired antiscorbutic properties when allowed to germinate.

Owing to the ease with which the vitamin C is destroyed in ordinary cooking or drying of fruits, vegetables, milk, and so forth, much interest has been manifested by several investigators in the study of the vitamin potency of various natural foods in the untreated condition and when subjected to varying degrees of heat treatment. Special interest has centered on the vitamin content of fresh milk, powdered milk, and pas-

teurized milk. There seems to be no doubt that pasteurized milk is nearly devoid of vitamin C, but it is asserted that certain milk powders still retain a demonstrable amount of this substance. It has been conclusively shown by Dutcher and co-workers (1920) and by Hart and co-workers (1919) that the vitamin C content of milk is dependent on the food of the cow, so that summer milks from cows on green feed are much richer in vitamin C than winter milks. Since there is such great variation in the vitamin C content of fresh milk, it hardly seems worth while to emphasize to the extent that has been done the importance of preserving the antiscorbutic properties of milk. It seems best to look to other articles in the diet to supply this principle.

Vitamin C is present in all fresh fruits and fresh vegetables. The tomato and the citrous fruits appear to be richest in it, and apples, grapes, pears, peaches, and other of our commoner fruits appear to be among the poorest of the fresh foods in vitamin C. Cabbage, lettuce, celery, and carrots are all excellent sources of it.

This is the most unstable of the vitamins. It is especially sensitive to oxidation, but recent investigators have demonstrated that it is much more stable at high temperatures in the absence of oxygen than was formerly supposed.

A most interesting observation with respect to vitamin C was made by Kohman and Eddy. They have apparently solved the problem of canning fruits and vegetables so as to preserve the vitamin C content. Taking advantage of the knowledge that fruits and vegetables when harvested are living and respiring structures, they (1924) have so treated

these articles as to use up the oxygen in the tissues before applying heat for the canning process. Apples contain about 5 per cent by volume of oxygen, and, as in other fruits, the process of respiration goes on. When the supply of oxygen is cut off the oxygen in the apple is used up in approximately thirteen hours. The apples are prepared for canning by peeling and quartering, and are then covered with a weak solution of salt in water to prevent access of air. The oxygen in the fruit is rapidly consumed and the fruit can then be canned or cooked in any way which seems desirable without loss of vitamin C. Apples prepared in this way and stored for nine months showed no appreciable loss of vitamin C. Since apples when held in cold storage gradually lose their vitamin C content, canning by this method actually preserves the vitamin much better than when the fruit is kept in its natural condition. Canned spinach nine months after canning was shown to be one of the richest sources of vitamin C yet found. They report that feeding tests with cabbage processed by a commerical method of canning showed a loss of vitamin C which is markedly less than that of cabbage cooked in open kettles. The loss is due to oxidation. Temperature and time of heating were not found to be factors.

Bezssonov (1922) is the only investigator who has yet attempted to prepare concentrated preparations of the vitamin C. He subjected cabbage to hydraulic pressure and immediately treated the expressed liquid with neutral lead acetate. The filtrate from this precipitate was evaporated to dryness under diminished pressure at 35° F. From each

100 c.c. of the juice he obtained 2.5 gm. of a slightly yellowish hygroscopic powder which contained 33 to 45 per cent of reducing sugar and 52 to 65 per cent of total sugar, as well as about 7.5 per cent of ash. This substance, free from fats and proteins, was active in protecting guinea-pigs in daily doses of 0.1 gm. Zilva (1923, 1924) has shown that the sugars can be removed from desiccated lemon juice by fermentation with yeast without destroying vitamin C.

Anatomic Lesions in Scurvy.—The two most noticeable conditions observable at necropsy are hemorrhage and fragility of the bones. The stomach, intestines, and cecum may show congestion, hemorrhage, or ulceration, but, according to Cohen and Mendel, these organs may in some cases be normal in appearance. These pathologic conditions are usually observed in animals fed oats and water or oats and milk, and less frequently in those fed the experimental diet of Cohen and Mendel. It is pointed out that an ever-present condition in advanced scurvy is lack of appetite. The food intake diminishes as the disease advances, but fasting does not produce the characteristic lesions of scurvy.

A remarkable observation made by Cohen and Mendel (1918) is that the swollen joints and tenderness often appear while the animals are still growing rapidly and have good appetites. Inanition can, therefore, play but a minor rôle, if any, in the production of the more prominent symptoms of scurvy. There seems to be regularly a period of ten days or so of fasting previous to the death of the guinea-pigs which are confined to a scorbutic diet of soy beans or cereals, for

the loss of weight during this period corresponds to the rate noticed in guinea-pigs during starvation.

LaMer and Campbell (1920) found an increase in the weight of the adrenal glands which they believe may represent a compensatory response to the decreased adrenalin production known to exist in the scorbutic animal. They believe that the heart and kidneys of scorbutic animals increase in weight, but that the liver is not enlarged.

Morel and co-workers (1921) state that the bone changes seen in experimental scurvy are not related to defects in calcium metabolism. Morikawa (1920) observed the following changes in the suprarenals in experimental scurvy in guinea-pigs: increase in weight, increase in lipoidal content of the cortex, and reduction in amount of doubly refracting fat. He states that the lipoid in the zona fasciculata was distributed in three layers; an outer layer rich in lipoids, a middle layer poor in lipoids, and an inner layer rich in lipoids. McCarrison (1921) has reported a remarkable enlargement of the suprarenal glands in the guinea-pig during experimental scurvy. The changes in these organs were slight during the first fifteen days. During this period the weight of the spleen, liver, stomach, and intestines was definitely reduced. These results have been confirmed by others. Wells (1921) says that the tooth pulp is abnormal in scorbutic guinea-pigs. Hess, Unger, and Pappenheimer (1922) found that prolonged exposure to direct light did not prevent scurvy in guinea-pigs. Gerstenberger and Burhans (1922) found that scorbutic infants and guinea-pigs, as well as polyneuritic pigeons, burned carbohydrates completely. Iwabuchi (1922) found

a decrease in the amount of ash in muscle and bone from scorbutic guinea-pigs. The fat content of the muscles and adrenals was decreased. The calcium content of the blood was 50 per cent below normal. Refractometric readings showed the protein content of the blood to be abnormal. Hemoglobin was markedly decreased, although the number of red corpuscles was essentially normal. Iwaduchi, however, could not confirm the observations of others that the calcium and phosphorus content of the blood was abnormal. Smith (1923) found no evidence of the deposition of calcium in the tubercle in infected guinea-pigs when calcium was administered together with cod-liver oil. He states that cod-liver oil has a definite but slight effect on the nutrition of the non-tuberculous guinea-pig.

Liotta (1923) found that there was a marked diminution in the number of red corpuscles, as well as in the amount of hemoglobin, and a slight increase in the number of leukocytes of the blood of guinea-pigs in scurvy produced by a diet composed exclusively of oats.

Höjer (1924) has discussed at length the pathology of the bones, teeth, muscles, heart, liver, spleen, kidney, adrenal and salivary glands, lung, blood, blood-vessels, and connective tissue in scurvy in guinea-pigs.

It is not easy to evaluate these observations because the guinea-pig is such an unsatisfactory animal with which to conduct any sort of experimental study. The diets employed to produce experimental scurvy in guinea-pigs have almost always been unsatisfactory in some respects other than a deficiency of vitamin C, and so one must qualify any state-

ments respecting the pathologic lesions resulting from deprivation for vitamin C, since it is not certain that other factors may not have contributed to produce these lesions.

For the benefit of those who are accustomed to regard an individual as well until clinical recognizable symptoms are apparent, it is appropriate to mention that Dr. James Lind, who in 1747 wrote his classic account of scurvy, in discussing scurvy in sailors made an observation which deserves our careful consideration today in relation to the effect which the diet may exert on the general well-being and behavior. He says: "An uncommon degree of sloth and laziness which constantly accompanies this evil (scurvy) is often mistaken for the wilful effect of the patient's disposition. This may prove fatal to many, some of whom when obliged by their officers to climb up the shrouds have been seen to expire and fall down from the top of the mast."

The cause of scurvy is now so well understood that there is little reason why it should ever attack masses of people except under extraordinary conditions of war and famine. The prevention of scurvy in infants is, however, a matter of importance, since the infant fed solely on heated milk supplemented with any list of foods not including fresh, unheated fruit juices, is likely to develop the disease within a few weeks. There are many still who do not recognize it. The wisest policy is to examine the diet of the child to see whether it has the antiscorbutic principle rather than to rely on clinical observations to determine whether it is in special need of vitamin C. The safe course is to give every child a suitable antiscorbutic fruit juice, such as orange juice or tomato juice.

It has been definitely shown that man, monkeys, and guinea-pigs develop scurvy when deprived of vitamin C, but it is certain that the rat, prairie dog, and apparently also birds are immune to it. This immunity has been shown in the case of the rat to be due to an ability on the part of this species to produce synthetically the vitamin C. This is demonstrated by an abundance of vitamin C in the livers of animals which have been deprived of the vitamin during the entire period of their growth. Such livers are highly effective in the cure of acute scurvy in guinea-pigs.

VITAMIN D

The experimental studies on rickets during the last few years constitute the best illustration which nutrition studies have yet produced of the manner in which animal experimentation can solve certain medical problems. Once a pathologic condition is produced experimentally in an animal, either by accident or design, if it be due to defects in the dietary, the solution of the problem and the correction of the dietary fault can be undertaken with the certainty that success will be the reward of effort. This follows from the completeness of our present-day knowledge of the essentials of a complete diet and of the great body of dietary information which we possess concerning the quality of many natural foodstuffs with respect to each of the essential nutrient principles. Experimental diets can now be planned which have any defect or defects desired, and which are known with reasonable certainty to be complete in all other respects.

Glisson (1650) published the first adequate description of a disease which is still commonly known as rickets. The condition had appeared in parts of England about thirty years previous to his writing. Later Barlow (1894) described the relationship of infantile scurvy and rickets.

Rickets is a disease which affects the entire body, although the most noticeable signs of it are seen in the bones. At the beginning of the disease the children are usually constipated. They are restless and irritable, usually apathetic and disinclined to play. They sleep poorly. Frequently a rachitic child rolls its head about on the pillow until the hair is worn off from the back of its head. The child perspires freely, especially about the head.

The muscles are lax and the tendons and ligaments may become elongated. Because of this, as well as from the softening of the bones, children do not walk or stand at the proper time. They do not profit by their opportunity for exercise like the normal child. The muscles of the intestine are affected as well as those of the legs and arms. Owing to weakness of the intestinal musculature and the muscles of the abdomen a pot belly develops.

As the disease advances deformities of the bones begin to appear. One of the first of these is the "rachitic rosary" or a line of knobs on the side of the chest where the bones of the ribs join the cartilages. The walls of the chest are drawn inward every time the child breathes, and grooves are formed along either side of the line of attachment of the diaphragm. The chest becomes compressed from side to side and "pigeon breast" deformities develop. Bosses of new bone are formed

on the side and front of the skull, and the head acquires a square shape. The ends of the long bones of the extremities become enlarged. The legs become knock-kneed or bowed. The bones of the arms bend, and there is marked enlargement of the epiphyses at the wrists and ankles. In severe cases curvatures of all sorts appear. Some children show very severe anemia. Some manifest extreme nervousness and often convulsions. Rickets is seldom fatal, but often the child dies from some complication, especially bronchopneumonia.

Rickets is in great measure confined to civilized races and to the temperate zone. The reason for this has long been a puzzle. Wild animals almost never suffer from rickets, and we now know that under domestication rickets is always associated with defective diet.

Experimental Rickets.—Mellanby (1919) produced conditions which he believed to be rickets by confining puppies to a considerable number of faulty diets. He did an immense amount of work, but apparently was unaware of the various factors which are essential for an adequate diet. His experimental work was not planned with a definite understanding of the dietary properties of the foodstuffs which he used. This is evident from statements like the following: "The greater the amount of bread eaten, the greater will be the tendency to rickets." "Whether, therefore, the protein effect depends on its own action remains to be decided. If the antirachitic effect of protein is established we will be able to comprehend one reason why milk is a better preventive of rickets than the corresponding amount of butter."

Mellanby was, however, the first to associate a group of

fats rich in vitamin A with the prevention of rickets. Whereas some of his data were suggestive that vitamin A was essential for bone growth, some of his results indicated that perhaps another factor was needed which was not identical with vitamin A. His experimental work did not prove or disprove this point. This is true for the reason that in many cases Mellanby did not appreciate the importance of the calcium to phosphorus ratio in his study of rickets. His experiments brought to light the following facts: Cod-liver oil possesses great antirachitic potency; suet and butter fats are also effective in producing calcification, whereas lard has no rachitic effect; peanut oil is the most effective of the vegetable oils. Cod-liver oil was found to be much superior to butter fat in preventing rickets.

The therapeutic value of cod-liver oil in rickets had long been accepted by many, but the nature of the curative substance had not yet been recognized. It is not surprising that Mellanby emphasized the fact that those fats especially potent in preventing rickets contained a high content of the vitamin A, and he was led to associate a lack of this vitamin with the disease.

Although Mellanby's studies are open to obvious criticism, nevertheless his experiments constituted pioneer work. Since the dietary requirements of the dog are not satisfactorily worked out, it is doubtful whether any one could have planned experimental diets which were as clean cut and decisive as those which have been used with the rat.

When Mellanby's studies were reported, a co-operative investigation between the Department of Pediatrics of the

Johns Hopkins Hospital and the Department of Chemical Hygiene of the School of Hygiene and Public Health, Johns Hopkins University, was in progress. Our program included a detailed study of the effects of diets faulty in specific ways on the growth and structure of the bones, and also studies on the composition of the blood with respect to its inorganic elements.

A notable advance in our knowledge of the etiology of rickets came in the spring of 1921. In March of that year Sherman and Pappenheimer published an important paper demonstrating that rickets could be produced in rats on a diet low in phosphorus, and that it could be prevented by the addition of alkaline potassium phosphate. Shipley, Park, McCollum, and Simmonds reported in May similar experiments. They reported the production of rickets by means of two diets. Both diets were deficient in vitamin A and phosphorus. When a complete salt mixture replaced the sodium chlorid and calcium carbonate the diets no longer produced rickets, but typical osteoporosis. These observations showed the importance of the inorganic constituents of the diet, especially of phosphorus, in relation to the disease. With the exception of Mellanby, who had placed but little importance on the salt composition of the diet, almost all other investigators have sought to produce rickets by a reduction in the calcium.

Our experience has shown that a diet of cereals and leguminous seeds, with casein 10 per cent and calcium carbonate 3 per cent, induced rickets in rats. We fed another group of animals this diet, but increased the casein to 20 per cent,

leaving the calcium carbonate at 3 per cent. These animals grew well and had normal bones. We had at this time made the observation, as had also Sherman and Pappenheimer, that the phosphate ion under certain conditions would protect against rickets. Since casein is a phosphorus-containing protein, we decided to replace it in this diet by the non-phosphorized proteins gelatin and wheat gluten, leaving 3 per cent of calcium carbonate in the diet as before. Diet 3,143 has the following composition: wheat, 33.0; maize, 33.0; gelatin, 15.0; wheat gluten, 15.0; sodium chlorid, 1.0; and calcium carbonate, 3.0 per cent. Since the diet contained considerable amounts of vitamins A and B, and proteins of good quality, the animals grew and remained in a fairly good state of nutrition for varying periods, depending on the season of the year. After about forty days on this diet we noted that the animals did not have full control of their hind legs. They had a peculiar gait due to a partial loss of control of the posterior extremities. The gait was tottering and the hind quarters wavered slightly from side to side. When the animal started to move off rapidly it hopped, usually favoring one hind leg. This diet always produces a florid condition of rickets, and calcification of the cartilage is wanting. The picture of rickets is much more exaggerated than that seen in human beings. If this diet is fed with 2 per cent of calcium carbonate instead of 3 per cent, the histopathologic picture is identical with that seen in the rickets of children.

Diet 3,143 produces the following changes in the bones of rats: the bones are very soft; the ends are much enlarged;

the enlargement of the ends of the long bones is due to an increased depth and probably also to an enormous metaphysis; the cartilage in every animal is entirely free from lime salt deposits; some irregular lime salt deposition is sometimes present in the metaphysis of certain animals; the cortex is thin and composed of bone with scanty central cores of calcified material having broad osteoid borders. The breadth of the osteoid borders is extreme in these animals. Few osteoblasts, or at least cells which could be identified as such, could be found lining the osteoid trabeculæ. The evidences of resorption of the calcified portions of the trabeculæ were slight.

Animals which show this wide metaphysis free from calcification are ready to be used for determining the presence or absence of vitamin D in foodstuffs. They must at this point be kept in individual cages in order that the food consumed can be recorded. McCollum, Simmonds, Shipley, and Park (1922) demonstrated that animals in this condition will produce a positive "line test" if allowed to starve. Howland and Kramer (1922) showed this calcification to be accompanied by changes in the calcium and phosphorus in the serum. They found the serum of rats on diet 3,143 to contain about 10.5 mg. of calcium and about 3 mg. of phosphorus per 100 c.c. of serum. During starvation the phosphorus increases to three or four times this amount. This produces the healing seen in starvation of these rachitic rats. In testing animals for the antirachitic properties of foodstuffs it has been our custom to examine the distal end of the left femur and the proximal end of the left tibia for re-

formation of the provisional zone of calcification. The bones are split in two with a sharp scalpel and one-half of each is fixed in 10 per cent formaldehyde, decalcified in Müller's fluid and embedded in parloidion. The other half of each bone is immersed in 1 per cent silver nitrate and exposed to the sunlight or to the light of a Mazda lamp and studied through a binocular microscope.

Rats which gave a positive "line test" differ from the controls in having a broad linear deposit of calcium salts on the metaphyseal side of the epiphyseal cartilage. The band, which may not be complete, is separated from the shaft of the bone by the depth of the metaphysis and from the nucleus of ossification of the epiphysis by the depth of the epiphyseal cartilage. It can be seen on a freshly cut surface of an untreated bone as a yellow line which marks the epiphyseal border of the metaphysis. The deposit is blackened by 1 per cent silver nitrate in gross specimens. It appears like a cross section of a black honey-comb when it is examined with a binocular microscope. The metaphysis of these bones usually appears to be congested. The calcium salt deposit is in the proliferative zone of the cartilage. It may extend completely across the bone or may be interrupted or fragmentary according to the activity of the calcium-depositing substance. It is stained brown in permanent sections by silver nitrate, or an intense blue by hematoxylin. Rats fed 2 per cent of cod-liver oil for five days give a positive line test.

Although we presented evidence (1922) which was all but conclusive that there is in cod-liver oil and butter fat a calcium-depositing substance distinct from vitamin A, it could

not be looked on as finally proved. Our experience with diets very low in calcium, but containing cod-liver oil or butter fat, demonstrated that 1 per cent of cod-liver oil was very superior to 20 per cent of butter fat with these diets. The possibility remained that butter fat and cod-liver oil contained one and the same substance (vitamin A), but that cod-liver oil contained much more of this. Zilva and Miura (1921) stated their belief that cod-liver oil was 250 times as potent as butter fat as a source of vitamin A. Our experience had convinced us that existing methods were incapable of differentiating beyond doubt between vitamin A and a special calcium-depositing substance, should this exist. We formulated a plan which involved a comparison of a selective list of fats in respect to three kinds of effects in nutrition: (1) We tested cod-liver oil, shark-liver oil, butter fat, and several vegetable oils for their potency in causing the cure of xerophthalmia due to the lack of vitamin A; (2) we made comparative tests of the same fats to determine their value in promoting growth in young rats which were restricted to a diet so low in calcium that satisfactory growth was not possible without the provision of some substance which would make for greater efficiency in the utilization of calcium than that which could be effected in its absence; and (3) we further studied these same fats by means of our "line test" to discover their relative values for inducing the deposition of the line of calcium salts in rachitic bones. With the data secured from these three distinct types of test we were able to interpret accurately the results of much of the confusing experimental data in the literature.

Space will not permit of a detailed exposition of these experiments. It must suffice to refer to our original papers and to a somewhat detailed account which will be found in the third edition of my book, "The Newer Knowledge of Nutrition."

Two to 3 per cent of fish-liver oils or of butter fat were found to effect a prompt cure of incipient xerophthalmia under the conditions of our test. On the other hand, 8 to 20 per cent of various vegetable oils failed to cure the eye condition after it had once developed. Special mention should be made of the fact that 15 per cent of cocoanut oil did not cure or prevent xerophthalmia. Cocoanut oil was the one vegetable oil which we studied which exerted a slight but demonstrable effect in causing the healing of the rickets lesion.

Hopkins (1920) was the first to point out that oxidation destroys vitamin A. He showed that if oxygen was allowed to pass through heated butter fat the vitamin A was readily destroyed. With this treatment butter fat lost its power to induce growth or to cure ophthalmia of dietary origin. Mellanby (1921) attempted to make use of this means of destroying vitamin A in order to determine whether there is a distinct antirachitic substance. He found butter fat of little value for protecting against rickets after it had been oxidized, whereas cod-liver oil after the same treatment, that is, heated to 120° C. for four hours while oxygen was passed through it, still protected his animals against rickets. He stated, "If it should happen that four hours' heating and oxidation at 120° C. also leaves a large amount of fat-soluble A in cod-liver oil, it will go a long way, especially when con-

sidered together with the butter results, to clinch completely the identity of fat-soluble A and the antirachitic vitamin." Mellanby used no method of testing for vitamin A as distinct from the calcium-depositing substance, since he did not make use of the ophthalmia test.

We found that cod-liver oil treated with a stream of air bubbles at a temperature of boiling water for twelve or twenty hours no longer contained sufficient vitamin A to relieve rats from xerophthalmia when administered to the extent of 2 per cent of the diet. Untreated cod-liver oil under these conditions invariably causes complete recovery within five days. Two per cent of fresh butter fat under exactly comparable experimental conditions effects the disappearance of ophthalmia within five to ten days.

We found 2 per cent of oxidized cod-liver oil very effective in causing the healing of the lesion of rickets, notwithstanding the fact that its potency for the cure of ophthalmia had been lost. Since it is possible to destroy by oxidation the vitamin A content of cod-liver oil and still preserve its antirachitic potency, we interpreted our experimental data as showing that the antirachitic substance is distinct from vitamin A.

Cod-liver oil is said by Darbey to have been used by physicians at the Manchester Infirmary in 1789, but Guy (1924), who has made a study of the whole literature of cod-liver oil, states that its recognition as a curative agent cannot be traced to any person, time, or place. The merits of cod-liver oil as a preventive or curative agent in rickets is now known to be due to its high content of the substance to which the name vitamin D has been assigned. No adequate explanation was

available for the absence of rickets in tropical regions, but the last few years have seen the accumulation of convincing evidence that sunlight, like cod-liver oil, exerts a protective influence on the metabolism.

Zucker, Johnson, and Barnett (1922) report the production of rickets in rats on a diet containing an excess of base over acid. When they substituted calcium chlorid for calcium lactate in equivalent amounts they increased the acidity of the diet. The bones of rats on this diet were nearly normal or showed mild rickets. The addition of 2 per cent of ammonium chlorid, they stated, prevented the development of rickets. They believe that a diet which, from the point of view of balance between calcium and phosphorus, should not lead to rickets, may do so when there is brought about a situation to which the acidity of the intestinal tract is lessened.

Pappenheimer and co-workers (1922, 1923) have discussed the effects of varying the inorganic constituents of a rickets-producing diet. Inorganic salts other than calcium or phosphate seemed to be without influence on the development or prevention of rachitic lesions.

Bosányi (1924) has reported remarkable experiments in which he showed the subcutaneous administration of aqueous extracts of normal bone-marrow causes the healing of rachitic bones in the rat. The phosphorus content of the extracts was very small, so the effects could not be attributed to the element. He believes that the failure of the initial deposition of calcium in rickets is due to the stoppage of the biological functions of the bone-marrow through which nor-

mally a permanent deposition of calcium is insured. Normal functioning bone-marrow produces a substance which is transmitted to the tissues requiring to be calcified which renders them capable of calcification. This biologic factor in an active state can be extracted with water from the healthy marrow, but this is not identical with the enzyme present in the watery extract. His experiments demonstrate that this factor is not produced in the marrow of rachitic rats.

The fat melted from bone-marrow had a slight protection if melted from the red bone-marrow, but no effect if from yellow marrow. The marrow wholly defatted protected animals against rickets. When filtered through collodion, aqueous extracts of marrow had no antirachitic effect. Dialyzed extract had no effect, whereas the gelatinous remainder not dialysable had a markedly antirachitic effect. Heating to 70° C. did not affect the properties of the extracts, but higher temperatures destroyed it. Extracts were made of liver, spleen, thymus, thyroid, and pancreas, of which the spleen extracts only were found to be antirachitic. Extracts of the spleen of a rachitic animal were inactive. Extracts of bone-marrow of rachitic rats which had starved from four to five days and showed healing were not antirachitic. The substance is present only in normal bone-marrow. Bosányi employed the "line test" procedure.

Bethke, Steenbock, and Nelson (1923, 1924) raised the question as to whether or not some of the beneficial results recorded in the literature on feeding of calcium salts may not have been due to mass action of the calcium counteracting the effects of vitamin deficiency. They found where no fat-

soluble vitamins were added the calcium of the blood was constantly depressed, but apparently the greatest depression took place when the most phosphate was added.

Zucker, Pappenheimer, and Barnett (1921) stated that the antirachitic principle is found in the ether-soluble, unsaponifiable fraction of cod-liver oil after alkali hydrolysis. This we have ourselves observed. The fatty acids of cod-liver oil are entirely inactive in curing rickets. Zucker (1922) reported that a good yield of a crude product of the antirachitic vitamin could be obtained by extracting cod-liver oil with 95 per cent alcohol. The mixture of fatty acids in a small amount of oil and other substances was saponified with NaOH. The calcium soaps were then precipitated and ultimately extracted with acetone. The acetone extract is exceedingly potent.

Howland and Marriott (1918) discussed the theories as to the causation of infantile tetany. Tetany has been referred to: (*a*) Dysfunction of the parathyroid gland; (*b*) the character of the food; (*c*) intoxication by calcium; (*d*) intoxication by guanidine, or methylguanidine; and (*e*) lack of calcium. They stated that the evidence in 1918 failed to support most of these theories, although there had been in the literature for a hundred years satisfactory descriptions of infantile tetany. They pointed out that normal calcium of the blood is 10 to 11 mg. per 100 c.c. of serum. In some cases of rickets they found a lowering of the calcium to 8 mg., although in some cases of rickets the calcium remained normal. In tetany during the active symptoms the calcium content of the serum was invariably reduced and may fall as low as

3.5 mg. In convulsive disorders other than tetany they found no reduction of the calcium of the serum. They found calcium chlorid given by mouth caused prompt relief of spasmodic symptoms. Howland and Kramer (1921) reported studies on the determination of the inorganic phosphorus of the serum. Their results represent the orthophosphoric acid content of the serum, the only form of phosphorus which can react with calcium to form tertiary calcium phosphate. They believe there can be no doubt that the inorganic phosphorus represents a definite chemical entity, since it is present in nearly constant amounts in the same individual as well as in normal individuals of the same age. Their studies show that in rickets there is a marked decrease in inorganic phosphorus in the serum. To this deficiency they ascribed the failure of calcium deposition. Non-rachitic infants and young children have between 10 and 11 mg. of calcium and about 5 mg. of inorganic phosphorus per 100 gm. of serum.

We found (1922) that a pathologic condition corresponding in all fundamental respects to rickets in human beings can be produced by diet in two ways: by diminishing the phosphorus and supplying an optimal or an excess of calcium, and by reducing the calcium and maintaining the phosphorus at a concentration somewhat near the optimum. As a result of these experiments we were led to believe that there are two kinds of rickets, one being characterized by a normal or nearly normal blood calcium and a low blood phosphorus (low phosphorus rickets; calcium to phosphorus ratio large); the other being normal or nearly normal blood phosphorus with a low blood calcium (low calcium rickets; low

calcium to phosphorus ratio). The investigations of Howland and Kramer (1921) and of Kramer, Tisdall, and Howland (1921) on the calcium and phosphorus content of the blood serum in rickets and tetany supported this view. These observers found that in children suffering from rickets alone the phosphorus of the blood serum is low and the calcium about normal. In children suffering from tetany complicating rickets, on the other hand, the calcium is low, but the phosphorus not far from normal. They believe that tetany is an expression of the nervous tissues of an insufficiency of the calcium ion, and that rickets is essentially an expression on the part of the skeleton of disturbed relations between the calcium and phosphate ions of the body fluids. They stated that tetany is frequently associated with rickets because rickets is a disease in which the calcium ion in the body tissues and fluids is subject to variation. Tetany occurs independently of rickets just as rickets occurs independently of tetany. Since tetany may occur with a low phosphorus form of rickets it does not serve to mark off one form of rickets from the other. Tetany is essentially associated with a low calcium form of rickets and, for all practical purposes, the low calcium form of rickets is the rickets of tetany.

Howland and Kramer (1923, 1924) found that in uncomplicated cases of rickets the concentration of calcium in the serum is normal or nearly so, whereas the phosphorus concentration is regularly low, so that the product of the two concentrations is at or below 30. In tetany the product is also low on account of the striking reduction in the calcium. In all of these cases rickets was present. After cod-liver oil

or ultraviolet-ray therapy the phosphorus rises so greatly as to make the product two or three times what it was before. The graph taken from their paper was constructed from figures for the concentration of calcium and inorganic phosphorus both of children and of rats. This illustrates clearly the significance of the products of these concentrates. On the abscissa are marked the concentrations of phosphorus; the oblique lines represent the concentrations of calcium and the products may be read off from the ordinates. The cases of active rickets occurring in children are represented by a circle, and those in rats by a triangle. Healing is indicated by a dark circle or by a dark triangle. It will be noted that below the horizontal line corresponding to the figure 30 on the ordinate all the triangles and circles are light. Above 40 they are all dark. Between 30 and 40 only one is dark. This graph is to be interpreted that when the product of calcium and phosphorus figures is below 30, rickets is to be expected; between 30 and 40 it is impossible. When the product is above 40 either healing is taking place or rickets is entirely absent.

Petersen (1924) reported an experimental study of ununited fractures with special reference to the inorganic bone-forming elements in the blood serum. He applied the principles elaborated by Howland and Kramer, and by McCollum, Simmonds, Shipley, and Park in their studies on experimental rickets in the rat. He found it possible by dietetic management to lower the phosphorus content of the blood of dogs to a point where the product of calcium and phosphorus figures was less than thirty. He concluded from his

study that in the healing of fractures a definite relationship exists between the concentration of the inorganic bone-forming elements in the serum and the rate of repair. If the phosphorus and the product of the calcium times and phosphorus figures are again raised to their normal level, the fractured bones will unite. This condition he was able to establish by dietetic management.

The Effect of Light in the Prevention and Cure of Rickets.— There has long been a belief among clinicians that sunlight, calcium-rich food, and cod-liver oil are of value in the treatment of disease, especially rickets and tuberculosis. Palm (1890), as a result of topographic study of the incidence of rickets, believed that sunlight should be looked on as a therapeutic agent. Buchholz (1904) successfully treated rickets with artificial light. Neumann (1909) observed that rickets and tetany did not occur at high altitudes in Switzerland, but was found at lower altitudes where the sunlight was less intense. In 1912 Raczynski wrote: "It is the sun which plays the principal rôle in the etiology of rickets." He gave the first proof of the favorable influence of light on mineral metabolism by an experiment on puppies. One puppy was reared in the dark, the other in sunlight, and both were suckled by the mother. At the end of six weeks he found the calcium and phosphorus in the body of the puppy reared in darkness was very much less than that of the one reared in sunlight. Commenting on his studies he says, "It is possible to assume that the lack of action of sunlight, by influencing in so unfavorable a manner the assimilation of calcium oxid in the young organsim, is one of the causes of rickets."

Huldschinsky (1919) reported that the ultraviolet ray exerted a curative action on rickets. Winkler (1918), Riedel (1920), Erlacher (1921), Mangert (1921), Hess and Unger (1921) extended these observations and established the therapeutic value of light. Sachs (1920, 1921) and Huldschinsky (1920) reported the cure of tetany with light treatment. Shipley, Park, McCollum, and Simmonds (1921) and Hess (1921) observed the prevention of rickets in rats by exposure to sunlight.

Hume (1922) found that irradiation with a quartz mercury vapor lamp prolonged the growth of rats on a diet free or almost free from vitamin A. She concluded that there is an interaction of light and vitamin A in the growth of rats, but no photosynthesis of the vitamin. Goldblatt and Soames (1923) irradiated rats for four weeks which were declining in weight due to vitamin A deficiency. Livers of these rats were fed to other rats on a vitamin A free diet and the latter responded with growth. They raised the question as to whether or not vitamin A was synthesized. Irradiated rats on a diet free from vitamin A do not continue to grow, although they do grow better for a time than rats not irradiated. However, they finally lose weight and die like those not irradiated. Since this is true, why did the livers of rats which were declining in weight, which were then irradiated, make other rats grow? They do not interpret their results as indicating a synthesis of vitamin A, but are unable to explain them.

Hume and Smith (1923) reported that when rats are fed a diet deficient in fat-soluble vitamins, but kept in glass jars

which have been exposed to the mercury vapor lamp for ten minutes every second day, they grew better than the controls not so treated. Rats exposed to air containing ozone, drawn over the quartz lamp and passed through 3 meters of glass tubing, showed poorer growth than the control animals. The same rats subsequently treated in glass jars filled with irradiated air gave some growth response. The growth of rats placed in irradiated jars from which the irradiated air had been displaced is not prolonged.

Webster and Hill (1923) were unable to confirm the observations of Hume and Smith. They also tried without stimulating effect: ozone, dilute NO_2, gas, air in glass jars which had been exposed to x-ray tubes and which was presumably ionized, and cigarette smoke. They found that exposure for thirty minutes daily to air led from the enclosure of the mercury vapor lamp failed to influence rickets in young rats on a deficient diet.

Steenbock and Black (1924) report the surprising observation that rat rations irradiated by the mercury vapor quartz lamp can be activated so as to make them growth promoting and bone calcifying to the same degree as when rats are irradiated directly. The activation takes place when the ration is irradiated in an open dish or in a stoppered Pyrex or quartz flask filled with air or carbon dioxid, but not in a brown bottle. The activation is not destroyed by subjecting the ration to a vacuum, heating it for forty-five minutes at 96° C., or letting it stand for twenty-four hours at room temperature. They confirm the observation of Goldblatt and Soames (1923) in finding that liver taken from irradiated rats

is growth promoting, whereas the liver from non-irradiated rats is inactive. The same was found true of lung and muscle tissue. Inactive muscle, after removal from the body, exposed to the radiations of the lamp, was found to have become activated, being both growth promoting and bone calcifying. Liver treated the same way also promoted bone calcification. The activity of liver taken from irradiated rats was not destroyed by drying at 96° C. for twenty-four hours and then keeping it in a stoppered bottle for two months.

Hess (1924) states that attempts to prevent rickets in rats by means of fluids which contain radium failed. Water to which radium bromid had been added was fed daily, but without effect. This was likewise true with regard to linseed oil to which minimal amounts of radium had been added. Subcutaneous injections in water were also without effect. He found that cottonseed oil when rayed for an hour with the mercury quartz lamp at a distance of 1 foot acquired antirachitic potency. Linseed oil was also made antirachitic by ultraviolet rays. Mineral oil irradiated in the same manner gave a negative result.

Hess and Weinstock (1924) observe that "while it is impossible to state definitely that the substance which is formed is the same as that which is responsible for the remarkable curative value of cod-liver oil, it has been found by means of chemical examinations that cod-liver oil can be separated into two distinct portions, one which is of value and one of no value in curing rickets. . . . Vegetable oils which have been exposed to the ultraviolet rays can likewise and by the same chemical means be separated into a portion which is

of no value, and another which acts as a specific in warding off or curing rickets. It would, therefore, seem that a substance has been formed in the vegetable oils by the action of the ultraviolet rays similar to that which is naturally present in the liver of the cod."

VITAMINS AND REPRODUCTION

Evans and Bishop (1922) have reported studies on the relation between nutrition and fertility. They point out that animals confined to a certain ration are usually sterile, but not invariably so. Ovulation is unrelated to the reproductive function on this basic diet, since the ovulation rate is normal, but there is failure of the females to reproduce. The animals conceive and the placentæ are implanted. In other words, early steps in reproduction are not interfered with, but about the fourteenth day after a positive mating the majority of the females give what Evans and Bishop designate as the "placental sign." This consists of a slight leakage of blood from the placenta. The vaginal smear contains blood cells due to a leakage of the placenta. Necropsies performed at this time show that the sign has been given by the placentæ, which are very abnormal. In some instances the fetuses have been completely absorbed. They pointed out that when certain natural foods, for example, lettuce, are given to a female, even after one or more resorptions have taken place, she will produce normal young. In summarizing their extensive studies on the production of sterility with nutritional regimens they state that the basal ration, which was composed of casein, lard, and cornstarch,

with salts, supplemented with yeast as a source of the vitamin A, failed to give normal fertility in a large number of female rats. They have demonstrated that this sterility may be prevented or cured after its appearance in any individual case by the addition of certain natural foodstuffs to the basal ration. They found that lettuce, meat, whole wheat, wheat germ, rolled oats, dry alfalfa, and large quantities of milk fat will relieve this condition. Wheat germ oil is said to be especially effective even in very small doses. Whole milk, fresh or dried, cod-liver oil, orange juice, and yeast failed to act as curative agents when added to the basal diet. The unknown fertility-conferring factor which they have tentatively called "X," shown to be present in the above list of foods, cannot be identified with the known vitamins A, B, C, or D. Sure (1924) has confirmed the observations of Evans and Bishop relative to the potency of the ethereal extract of yellow corn, wheat embryo, and hemp seed in preventing sterility in the rat. He also observed fertility when commercial cottonseed oil and commercial olive oil were introduced to the extent of 5 per cent into the sterility diet, but not with commercial cocoanut, linseed, or sesame oil.

CONCLUSION

This, then, is the story of our present knowledge of vitamins, and the rôle they play in influencing metabolism. Of necessity the account has been shorn of all details. The field of vitamin research, both on the chemical and pathologic side, is still a fertile one, and within recent years has attracted an ever-increasing number of investigators. Some of the

work recorded is probably open to criticism on the basis of faulty dietetic management of the animals, but considerable time will be necessary in order to separate fact from error. It is still too early to attempt any general discussion of this kind, or a critical examination, especially of the more recently recorded observations.

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THE RELATIONS BETWEEN FERTILITY AND NUTRITION*

HERBERT MCLEAN EVANS

As is well known to most members of this audience, the science of nutrition, which may be said to have received its classical formulation by the lineage of investigators represented by Lavosier, Liebig, von Voit, Rubner, and many others, has undergone a striking enlargement in recent years by the discovery of the nutritive need of the body for very minute quantities of several kinds of chemically unknown substances, the vitamins. A summary of our knowledge in this field by one who has himself played a significant part in delineating it (Professor McCollum) has already constituted one of the lectures in this series, so that I can the better dispense with further elaboration here.

The discovery of the indispensability of these substances may be said to have come especially through efforts to rear animals on so-called pure or synthetic foods. The work was undoubtedly facilitated by the use of a hardy small mammal, sufficiently indiscriminate in its taste to reject little or noth-

* Part of this address is substantially identical with a report made on fat-soluble vitamin E to the National Academy of Sciences at their annual meeting, Washington, D. C., April 27, 1925 in conjunction with my associate, Dr. George O. Burr. It should, therefore, be borne in mind that Dr. Burr shares in every way in the elucidation of the latter problem.

ing edible, the rat. The occurrence of actual disaster, that is, death, when either of the two major vitamins, fat-soluble A and water-soluble B, are absent, made the detection of these two substances inevitable sooner or later. On the other hand, the experimental production of two grave, well-characterized diseases, scurvy and rickets, the former in guinea-pigs, has led with no less security to the recognition of C and D. I shall endeavor to present here in some detail the evidence which now establishes the existence of the fifth member of the vitamin class, the substance provisionally characterized by the symbol X, but for which one may use the serial designation E. Animals can suffer serious impairment, especially where it concerns some of the functions of the more hidden organs, without that impairment showing itself openly. It would consequently not seem too hazardous a conjecture for us to expect that refinement in our method of measuring the function of organs will lead to the discovery that the particular chemical work involved in the activities of some of the organs and tissues depends on still other specific foods. Indeed, one finds that twenty years ago this hazard had already been taken by Professor Gowland Hopkins, to whom in part we owe the opening of this field of research when he said, "The animal body is adjusted to live either upon plant tissues or other animals, and these contain countless substances other than the proteins, carbohydrates, and fats. Physiologic evolution, I believe, has made some of these well nigh as essential as are the basal constituents of diets."

PHYSIOLOGY OF REPRODUCTION OF RATS

A number of years ago we attempted in the Anatomical Laboratory of the University of California a very careful analysis of the steps in the physiology of reproduction in these small rodents in the hope of utilizing this material for experimental procedures in the field of mammalian embryology. Enough was learned, I feel, to constitute a very substantial advance on the previous indefiniteness of our knowledge in this interesting realm. As I hope to portray presently, it was possible for us to discover in the living animal the approach of œstrus and correct time of insemination, the time at which the final ripening or maturation changes occur (involving the formation of the first polar body) and the time at which follicular rupture or ovulation actually occurs and the tubal journey begins; furthermore, if mating was permitted, the detection of the state called pseudopregnancy and, finally, on the thirteenth to the sixteenth day, the definite detection of pregnancy through the discovery of what we may call the implantation sign. We had in our hands then, probably for the first time, an adequate method for the detection of aberrations in the mysterious processes involved in the physiology of reproduction. As a matter of fact, when we turned our attention to the possible dependence of reproduction on nutrition we were promptly struck by a host of new facts.

Relation to Nutrition.—Nutritive regimens adequate for livelihood, indeed, for approximately normal growth and for the appearance of health in animals, are still inadequate for the maintenance, for instance, of the normal rhythm of

ovulation. And when we turned to the study of reproduction we were astonished to find it fail in a large proportion of animals reared on pure foods and an abundance of the hitherto established vitamins, particularly A and B. Small amounts of some natural foods cured the disorder or sterility provoked by these regimens, a disorder which was hence a true dietary deficiency disease. Furthermore, the beneficial properties of these curative foods could be extracted from them and, in turn, other satisfactory extracts made from these extracts which are at this point very concentrated and need to be fed only in mere traces. We have hence, I believe, adequate evidence before us for declaring the existence of a fifth class or group of the vitamin substances: the substance fat-soluble E.

Before turning specifically to the data which bear on the discovery of the new vitamin, on its occurrence in various foods, on its physical and chemical characteristics, and the present stage of its isolation, it would seem best to prepare the ground, as it were, by reverting to our general studies on the reproductive system and to describe also briefly some of the other relations between fertility and nutrition, a field of which the vitamin studies constitute but a part and one which holds promise of important rewards to the investigator who will explore it with the criteria now available for advances in these newer aspects of mammalian embryology. That there are many relations between fertility and nutrition (some of them at present unconjectured) is, I believe, a fact.

Vaginal Changes During the Œstrual Cycle.—The precision

with which one may detect the periodicity in ovarian function in some laboratory animals is truly remarkable. Many years ago Morau, Lataste, Retterer, and Königstein described changes in the character of the epithelium of the genital tract and especially the vaginal canal in several common types of mammals. They made out definite changes, for instance, associated with the phenomenon of heat, or rut, but in 1917 Stockard and Papanicolaou showed that microscopic examination of the cell detritus and exudate in the vaginal canal (from samples which can be obtained with maximal celerity and ease in the living animal) enable us to determine the exact time of œstrus and ovulation and to segment the œstrual cycle into definite periods or steps. When we examined the rat in this way we had no difficulty in making out the existence of similar steps or periods in the œstrual cycle, which recurs with striking regularity every four or five days. In these forms the mucous membrane of the vaginal canal exhibits a period of growth, differentiation, and desquamation, the various steps of which take place in so orderly a sequence as to furnish us more information about the approach and incidence of ovulation than is furnished, for example, by the monthly appearance of a sanguineous discharge in the human being. In fact, cyclic changes affect the entire genital canal from the oviduct to the vaginal orifice. The ovary presides over or has some necessary connection with these changes, for on its ablation the changes disappear never to recur save on successful ovarian transplantation, when they may be again just as regular. We are even now in ignorance as to the exact time

relation between human menstruation and ovulation. The relation of œstrus to ovulation has been somewhat clearer, for the two events have always been recognized as close together in time. Our newer studies, however, inform us as to the exact time relations of these two phenomena, ovulation occurring at a precise time interval after œstrus. By killing animals at the various steps in what is an orderly progress of changes in the cellular character of the vaginal smear, it is possible to relate these changes with certainty to the time of ovulation. Immature animals, pregnant animals, or those from which the ovaries have been ablated do not, as has already been indicated, ever show these changes in the character of the vaginal smear, but, when ovarian follicles begin to grow and rupture, the cell changes invariably occur. Though we know many details about these changes, the essential facts are briefly as follows:

Irregularly shaped, small, nucleated epithelial cells mixed with leukocytes constitute the picture found in the resting stage. Preparatory to œstrus and ovulation there is a sharp pause or halt in the immigration of leukocytes which usually creep in in considerable numbers from the subjacent capillaries through the epithelial cells of the vaginal mucosa into the lumen. Leukocytes are suddenly no longer encountered in the smear. Epithelial cells alone, a peculiar type of them, sometimes in sheets, are now found. Quickly succeeding this change come non-nucleated, transparent cornified cells, sooner or later in similar sheets. A massive production of these cells takes place, so that macroscopically a cheesy detritus is found. Eventually leukocytes again

recur and quickly thereafter are found in enormous numbers. The cornified epithelial cells give way to scanty small nucleated ones. This is the cycle of changes. The presence of true cornified cells, singly or in sheets, is a reliable criterion for impending maturation of ova and consequent rupture of Graafian follicles in the ovary.

In the case of a large colony of animals, three persons working as a so-called diagnostic group can thus remove them from their cages, carry to the examining table and register on individual record cards the cell types found in every individual daily. The individual smears are quickly tapped into a drop of salt solution and the cell types floating there diagnosed almost at a glance with the low power of the microscope, the diaphragm being turned well down. In this way the œstrual cycles have been followed in the cases of every animal studied by us.

Effect of Vitamins A and B on Ovulation and Œstrus.— When, a number of years ago, animals were reared and maintained on a classic pure dietary mixture consisting essentially of casein, cornstarch, lard, butter, and salts, to which an appropriate separate dose of dried yeast was added daily for vitamin B, it was soon noted that while all other phenomena were apparently normal, the animals showed late maturity and infrequent ovulations. When the yeast dosage given these animals was greatly increased, for instance, from a daily quantity of 100 mg. to one of 400, 500, or 600 mg., the ovulation rate became normal, that is, ovulation recurred every four or five days. It was apparent, then, that we had a new and more sensitive test for physiologic well-

being than that furnished by either growth, glossy coats, bodily activity, or the other easily detectable signs.

When we came to study the situation with reference to vitamin A, equally interesting discoveries were made. As is well known to you, food impoverishment in this vitamin has for its sequel, sooner or later, trouble with the secretion of the lacrimal gland into the conjunctival sac and with the nutrition of the cornea, so that the occurrence of the so-called xerophthalmia is practically pathognomonic for deficiency of vitamin A. Nevertheless, it has long been recognized that xerophthalmia can be prevented, or at any rate greatly delayed, by satisfactory care of the eye, that it may be present in one eye alone, or that, indeed, it need not occur in animals when we are certain that death is due to what I have briefly designated as "A disease." Slowing and cessation of growth, and ultimate decline in weight also occur in these conditions, but they are not solely characteristic, of course, of this disorder. It was therefore highly interesting that in our attempt to study the sex cycles of animals impoverished in A, a new and characteristic sign of lack of this vitamin was quickly disclosed. We found that the tendency on the part of the vaginal mucosa to form cornified epithelial cells was no longer limited, as is normally the case, to the time of growth, maturation and rupture of the Graafian follicles, but that in A disease the desquamation of cornified cells was continuous and hence obscured all ovarian cycles that may also have been present. Furthermore, the new sign is not mediated through the ovary; that is, A impoverishment does not create a malfunction, as it

were, of the ovaries which thus continuously secrete hormones similar to those in œstrus, for the new sign is given in the presence of A disease even if the ovaries are ablated. It is not improbable in explanation of the new sign that we have to do with a disturbance of water metabolism and, in the case of the mucous membrane in question, again with xerosis. Studies on this point are in progress. I would only emphasize here the fact that the sign has been a valuable instrument in dietary research. It is given by no other food deficiency known to us save that of vitamin A, and in the study of many cases it has not only been found to occur with complete regularity, but to occur as early as any other sign, for example, decline in growth or ophthalmic disease, and in most cases earlier than other signs. Animals may be fed mixtures of the most miscellaneous foodstuffs, but serious deficiency in vitamin A will not be disguised by other inadequacies. By feeding subnormal amounts of vitamin A, ophthalmic disease may be prevented, and growth, in fact, kept practically normal, whereas deficiency in the substance A can still be frequently detected by the continuous exhibition of the new sign on the part of otherwise apparently healthy animals. Raising the level of A immediately abolishes the sign. Whether the sign is given in still slighter deficiencies in A (by cases, for instance, in which, as Sherman has shown, only lactation or longevity is affected) remains still to be shown.*

* Since the foregoing was written Wolbach and Howe have reported on the extensive transformation of epithelium in various parts of the body into a stratified, squamous keratinizing epithelium, calling attention particularly to the upper respiratory tract and to the renal pelvis, the

VITAMIN E

I shall now turn to the characterization of the sterility disease produced by pure foods or other dietaries lacking the substance which we have called fat-soluble E. When rats are reared on some "synthetic" food mixtures consisting of fat, carbohydrate, and protein in separate relatively pure form together with an appropriate salt mixture and the vitamins A and B,* they grow well and have every appearance of health. Depending somewhat on the exact character and the proportions of the constituents of the food, they sooner or later exhibit complete sterility. In many instances a transitory period of fertility, variable in length, follows the attainment of sexual maturity. This is usually the case with the male, but it is also frequently the case with the female. We are as yet imperfectly informed as to all the factors which may delay or prevent the onset of this peculiar form of dietary sterility which, as will be shown below, can be so spectacularly cured by small doses of vita-

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urinary bladder, and the seminal vesicles, epididymis, and prostate. The salivary glands and pancreas participate in the change. They do not note the change in the vaginal epithelium, the only epithelium from which samples may be removed with ease and at will in the living animal.

* Various proportions of casein, lard, butter, and cornstarch have been used, the commonest being that after Osborne and Mendel as follows: Casein 18, cornstarch 54, lard 15, milk fat 9, salts 4, 0.4 to 0.6 gm. dried yeast daily, the vitamin B being secured from daily administration of 0.4 to 0.6 gm. of whole dried yeast and A from the butter employed. In many instances, however, milk fat was omitted and the A requirements met with various levels of cod-liver oil which varied from a single drop daily to 2 per cent by weight of the ration. The salt mixture employed was after E. V. McCollum and consisted of NaCl 0.173, $MgSO_4$ (anhyd.), 0.266, $NaH_2PO_4 + H_2O$, 0.347, K_2PO_4, 0.954, $CaH_4(PO_4)2 + H_2O$, 0.540, citrate of iron, 0.118, calcium lactate, 1.300.

min E. The preliminary fertile period may, in fact, be very extensive, and it is this doubtless which has led some investigators to doubt the existence of a new specific vitamin essential for reproduction.* We may possibly with justification point to an analogy here with the regulation of inorganic metabolism and especially that of calcium and phosphorus by means of vitamin D, for it would appear from the researches of McCollum and others that it is the ratio between the inorganic elements in question rather than their absolute abundance in the diet which occasions disorder in ossification. It would appear that if the proper ratio is maintained animals will not develop rickets, but that, on the other hand, small amounts of vitamin D will enable animals to overcome very unfavorable ratios in the elements in question. In the case of young from females reared on a "pure" food ration, the

* We have found, for instance, that when lard is omitted from the diet, 5 per cent butter fat often suffices to confer fertility on the animals throughout the early portion, and in some cases the greater portion of the life span. With a dietary mixture of casein 18, cornstarch 73, milk-fat 5, and salts 4 we have had from twenty-six females a total of sixty-six litters, totaling 304 young, born during a time when litter-mate sister controls held on casein 18, cornstarch 63, lard 10, milk-fat 5, and salts 4 produced no young. It would appear from these facts that, although the actual amount of vitamin E which we know to be present (although low) in milk-fat must be the same in both of these diets, the lard exercises in some way a sterility producing effect. The explanation of such phenomena given by V. E. Nelson, that the higher fat quota alone is responsible for the sterility, cannot be maintained inasmuch as we have employed diets containing 24 per cent by weight of milk-fat with fertility invariably resulting. It is perhaps well to note that animals on the first or fertile ration, are not invariably fertile, and furthermore that while the majority are at first fertile, they also sooner or later tend to lose their fertility, and in those cases in which sterility supervenes, it is promptly cured by the administration of very small doses of vitamin E as hereinafter described.

young secured either from the early fertility period or by fertility induced by certain food extracts hereinafter described, we usually observe complete sterility from the very beginning of sexual life. We have abundant evidence that *sterility is a dietary deficiency disease, for it can be cured or prevented by a change in dietary regimen, a change involving the addition of certain single natural foods high in a new food factor, vitamin E,* or the addition of very much smaller amounts of extracts of these foods.* The sterility disease affects males and females differently.

In the male it eventually leads to destruction of the germ cells (eventually the entire seminiferous epithelium), but this is not the case with the female, where the ovary and ovulation are unimpaired throughout life, but where a highly characteristic disturbance occurs in gestation, namely, the death and resorption of the developing young.

"*Resorption Gestation.*"—It is necessary to insist on the peculiar character of dietary sterility thus produced in the female through lack of vitamin E, for it is only by ascertaining the existence of typical "resorption gestations" that one may be assured that he is dealing with deficiency in the specific substance E. Many other dietary deficiencies cause sterility in the female, but they all do so by interference with other steps in the reproductive mechanism than those involved in lack of E, usually by preventing œstrus, ovulation, fertilization, or implantation, but not by resorption

* Provisionally designated in previous publications from this laboratory as vitamin X. We now designate it E on account of its serial position following the alphabetic terminology proposed by McCollum, who named the antirachitic factor D.

after implantation has occurred. In order to establish female sterility as due to absence of fat-soluble vitamin E, it is necessary to establish with certainty the existence of œstrus and ovulation, coition, and implantation. Such information is best secured by use of the newer methods of studying the vaginal smear, by mating animals at the appropriate time in the œstrual cycle, by subsequent detection of the "bouchon vaginale" and residual sperm, of the cessation of cycles, and finally, on the fourteenth to sixteenth day of the occurrence of erythrocytes in the smear, a positive sign of implantation. In gestations where E is low or absent, the embryos seem at first normal, but sooner or later, often by the eighth day, retardation in development can be demonstrated. Evident abnormality, especially monstrosity, does not occur. At some time between the twelfth and twentieth day fetal death occurs, usually on the twelfth or thirteenth day, but for some days thereafter the maternal part of the placenta continues to live. There may also be continued gain in the mother's weight until the twentieth or twenty-first day. This would appear to speak decisively for peculiar need on the part of the developing young for the new vitamin as against placental injury as the cause of death. Furthermore, it would appear that the maternal placenta is not altered structurally sufficiently to justify the conclusion that its function had been impaired. Subnormality is seen not only in the embryo, but in the fetal parts of the placenta, both in yolk sac and allantois, especially in the former; in the yolk sac underdevelopment of the entodermal villi and blood islands is conspicuous, whereas, in the embryo one may note

impairment in the mesenchyme and its chief derivatives, the blood-vessels and blood-cells. The exact time of fetal death appears to vary in the case of individual mothers and, what is more remarkable, in the case of some embryos as contrasted with others in the same gestation. Thus dead and living young may occupy neighboring sites in the same uterine horn. Embryos may succumb shortly after implantation, or again only shortly before term.

Effect of Various Natural Foodstuffs.—Large numbers of females have been reared on various "pure" food regimens and bred shortly after the sixtieth day of life. Only those exhibiting a typical resorption were now employed to trace the distribution and abundance of the new food factor E in natural foods. Shortly after the incomplete or resorption gestation, a small amount of a single natural foodstuff was now added to the ration or fed separately from it, and the fate of the new gestation followed with similar care. In many instances a normal sized litter of vigorous young resulted. In others no alteration of the sterility was secured. We have thus charted the considerable and inconsiderable possession of E by common foods.

Distribution of Vitamin E.—It is present but never highly concentrated in a great variety of animal tissues, muscles, fat, and viscera, included in the latter being pancreas, spleen, liver, heart, hypophysis, and placenta. One of the most remarkable things about the content of E in animal tissues is the fact that the vitamin is low in the viscera. It is lower in the liver than in the muscles. A daily feeding of half the total liver of rats reared on natural foods will not provoke

fertility. There is failure also when the entire heart, spleen, brain, kidney, or testes are fed daily. The muscles and fat, on the other hand, while not a concentrated source of E, contain in their totality several times the minimal requirement for a successful gestation. E is present, but to a small extent, in milk fat. Nine per cent of this, which is included in our basic ration, together with 15 per cent lard, fails to prevent sterility, though with lard absent 24 per cent succeeds. Whole milk powder may constitute one-third of the ration by weight and sterility result. Yet when whole milk powder is the sole food, its fat content, 28 per cent, is sufficiently high to insure an adequate amount of E. There is definite evidence of a higher E content of milk given by cattle with access to fresh alfalfa pasturage. Cod-liver oil, though high in vitamins A and D, is notably lacking in E. Throughout the life of animals 9 per cent by weight of the ration may be constituted by cod-liver oil, a single drop of which daily is adequate for A requirements, and yet sterility results. In contrast with the paucity of E, even in its most abundant depots in animal tissues, is its concentration in the organs of certain plants, especially in seeds and green leaves. It can be demonstrated to be unhurt after careful desiccation of such leaves (lettuce, alfalfa, pea, tea). Thus in a series of experiments, 1.5, 1, and finally 0.25 gm. daily of the lettuce leaf powder proved efficacious in cures. The content of E is high in some cereals. We have found it in oats, corn, and especially wheat, where it is low in the endosperm, but concentrated in the embryo. The richness of wheat germ in E is extraordinary. We have found no other nat-

urally desiccated substance comparable to it in value; 250 mg. daily evokes cures. In the case of both wheat germ and lettuce leaf, ether extraction of the carefully desiccated substance removes E quantitatively and secures for us oils which are efficacious in daily, single drop (25 mg.) administrations. E is probably present in most commercial oils, so that when the latter constitute a high proportion of the diet, for instance, when fed as 15 per cent, displacing lard, fertility results. Such results have been secured with Wesson oil, cocoanut oil, olive oil. Cottonseed oil when hydrogenized constitutes the substance called Crisco. As is well known, it is practically devoid of vitamin A and has hence been frequently employed instead of lard in researches where an A-free diet was essential. Yet, when the fat content of our basic diet is represented by Crisco, fertility invariably results, this being, in fact, a curative agent. Crisco, cottonseed oil, corn oil, olive, cocoanut, walnut, peanut, and flaxseed oils can all be fed daily in quantities five times the required minimum of wheat germ oil without restoring fertility.

Proof of the Existence of Vitamin E in the Tissues of Animals Reared on Natural Foods and of Its Depletion in Those Reared on Synthetic Diets.—We have completed a series of cannibal experiments. Sterile females reared on "pure" food were killed daily, and their tissues (liver, muscles, and fat) fed to other females reared in an identical fashion and likewise of proved sterilty. At the same time normal females of proved fertility were similarly killed and fed to other sterile females reared on "pure" food. In all instances the tissues of rats reared on a natural food were able to

cause fertility in their sterile sisters. Of even greater significance would seem the demonstration that in no instance could a cure be obtained by the administration of the same tissues from sterile females.

The Survival of Fertility in Animals Shifted from a Diet Possessing Vitamin E to One Deprived of It.—If animals are reared on a diet of natural foodstuffs and, after their fertility is established, shifted to a pure food ration, they preserve their fertility for three or four months, and then lose it. Similarly, when sterile animals are cured with foods possessing the new vitamin, not only is the next gestation normal, but in some circumstances the next two or three gestations. The survival of normal fertility is roughly dependent on the amount of E in the curative diet. When by quantitative experiments we have determined the minimal dose of any "curative" food, that is, one capable of immediately restoring fertility, we have been able to see the immediate loss of this fertility in the next gestation on the pure food regimen.

Presence of Vitamin E in the Tissues of Normal Newborn Young.—Vitamin E is transferred from mother to offspring during intra-uterine life, for the tissue of newborn rats cures female dietary sterility.

Proof of the Normal Use or Wastage of Vitamin E in the Usual Metabolic Processes of the Body.—Groups of females have been reared on a natural food and their fertility established by trial gestations, after which they were all shifted to our standard pure diet. Half of them were bred immediately and in all instances were able to give birth to young in

the next two succeeding pregnancies, the third uniformly failing. As soon as the advent of sterility was demonstrated, presumably by the exhaustion of E due to the drain of the repeated pregnancies, the other half of the animals were bred. These sisters were by this time likewise sterile. This half had been shifted to the pure food at the same time as had the first half of the group, but had been shielded from the drain of reproduction and especially placental function. Hence it seems clear that the body stores of vitamin E are employed in normal metabolic processes at approximately the same rate, whether or not we have the drain of gestation.

An Excess of E Cannot Increase Fertility Beyond Normal Limits.—The administration to sterile animals of foods or extracts of foods known to be twice to twenty times as rich in vitamin E as is required for the birth of living young does not increase size or weight of the litter, or in other ways improve the performance of the reproductive mechanism beyond normal limits. This is in consonance with what we know of the action of other vitamins, there being little or no reliable evidence of advantage from an abnormally high quota of them, yet absolute need of the minimal quota and, for complete normality, a need of what we can call the effective quota.

Efficacy of a Single Curative Dose of Vitamin E Administered at the Beginning of Gestation.—Since the work which has previously been detailed showed a definite if transitory storage of E by the body, it seemed reasonable to suppose that a sufficiently high feeding of E on a single occasion (early in gestation) might suffice for that particular gesta-

tion. It was, in fact, found that success resulted from a single administration of the same total amount represented in twenty-two days of separate daily dosage with the minimal effective amount of wheat germ oil. The minimal effective daily dose was found to be about 25 mg., and a single administration of 550 mg. of the oil led, in all tests, to the birth of living young. Furthermore, curative foods or extracts of those foods can be fed as late as the fifth or sixth day of pregnancy and save the situation. Finally, the vitamin in the form of oil can be just as effectively administered parenterally (by subcutaneous or intraperitoneal injection) as by mouth.

Physical and Chemical Characteristics of the New Substance.—We come now to a consideration of the physical and chemical characteristics of the new substance. The vitamin may be called fat soluble, though its range of solubility is far greater than that of ordinary fats. While this range of solubility may really be due to the solubilities of impurities as yet associated with the vitamin, it is a fact that the most concentrated fractions yet obtained have been almost completely miscible with solvents representing such a range as methyl alcohol, ethyl alcohol, ether, pentane, benzene, acetone, ethyl acetate, carbon disulfid, and so forth. The vitamin is almost insoluble in water, yet we have repeatedly encountered its presence in water solutions. There is enough left in the water after precipitation of calcium soaps, for instance, to be extracted with ether and effect cures. The distribution ratio between water and ether is very large, for a few extractions with an equal volume of ether effect quan-

titative removal. This has been established by a large number of feedings of the non-saponifiable fraction, the residual soap always failing to produce fertility. The solubility of E in such substances as alcohol and pentane shows a large temperature coefficient and is so much greater than some of the contaminating substances, the sterols, for example, as to permit separation of the vitamin from them.

Vitamin E is remarkably stable to heat, light, air, and many of the ordinary chemical reactions. As regards temperature, while the ashing of wheat germ completely destroyed the vitamin, yet heating of the germ to 170° C. so that it was greatly charred left the E unimpaired. Distillation of wheat germ oil, or a fraction out of it, in superheated steam at 180° C. for several hours has not destroyed it. Distillation in vacuo up to 233° C. has not, in fact, caused any lowering of the potency of the fractions so treated, nor have any physical changes like changes in solubility been detected. We have not encountered evidence that daylight affects E in wheat germ oil, but there would appear to be partial destruction by exposure in thin layers to a powerful quartz mercury lamp for one hour. As regards oxidation, exposure of wheat germ oil for as many as twelve hours to a stream of air washed with acid and alkali, and at 97° C., has not destroyed E. At normal temperatures the vitamin is remarkably stable to both acid and alkali and many chemical treatments. It dissolves unchanged, for instance, in saturated alcoholic hydrogen chlorid. We have hydrogenized wheat germ oil in the presence of palladium at 75° C., and no injury to the vitamin resulted. Further, alcoholic extracts

of Crisco, a hydrogenization product of cottonseed oil, are always fairly rich in the vitamin. We have treated the germ oil with both 20 per cent hydrochloric acid and one-tenth normal acid for twenty hours at room temperatures without destruction of the vitamin. It is not destroyed by concentrated sulfuric acid. It resists the action of boiling 20 per cent alcoholic potassium hydrate, though partial destruction would appear to occur on very prolonged hot saponification. The saponification with 20 per cent alcoholic potassium hydrate can be carried out at 30° C. without great loss of the vitamin which goes into the non-saponifiable quota, 5 per cent of the oil, so that by this step alone a notable concentration of E is always attained. The non-saponifiable quota is, in turn, chiefly (73 per cent) sitosterol, which is largely insoluble in pentane in the cold, an excellent solvent for vitamin E, which, together with pigments and other materials, can thus be washed out of the sterols, leaving them white. The sterols are inactive. If the orange-red viscous oil obtained from the pentane is treated with methyl alcohol, more extraneous material is removed, and the vitamin goes into the alcohol portions which now can be mixed with petroleum ether or diluted to 90 per cent methyl alcohol, allowing an immediate separation into two layers, the petroleum ether invariably securing more of the vitamin, in fact, all of it, if the distribution be done with successive fresh portions of the petroleum ether. Further purifications can now be carried out both with digitonin, boiling methyalcohol, and finally, distillations in vacuo; yet the concentration effected, of course, does not relatively compare with

that effected with the first three steps of the procedure just outlined. The final yellow viscous oil does not develop crystals on long standing. It contains only a trace of ash and no nitrogen, sulphur, phosphorus, or halogen. It is remarkably potent. When 5 mg. are fed or injected under the skin of a female of proved sterility at the inception of a new gestation, normal litters of vigorous young are born and have been reared to adolescence. Sister control rats invariably continue sterile. Furthermore, the daily administration of only 0.3 mg. of this substance throughout the life of the male results in the retention of complete normality when animals are reared and held on pure foods, a normality proved by the weight and histologic condition of the testis and by weekly functional tests throughout a year, and controlled by the invariable development of sterility at the end of three months in litter-mate brothers held on the identical ration save for omission of the trace of vitamin.

OUTLINE OF FRACTIONATION OF 6 KG. OF WHEAT GERM

Six kilograms wheat germ.

Extracted with U. S. P. Ether in Soxhlet.

Ether extract. Active. Yield: 600 gm. 10 per cent.	Ether insoluble residue. Inactive.

Saponified in the cold with 20 per cent alcoholic potassium hydrate.

Nonsaponifiable matter (N. S. M.). Yield: 5 per cent. Contains all the active material.	Soaps and glycerol. Inactive.

Crystallized from cold pentane.

Pentane-soluble red oil. Yield: 33 per cent of the N. S. M. Contains all active material.	Pentane-insoluble solids. Sitosterol. Yield: 66 per cent of N. S. M. Inactive.

Extracted with hot methyl alcohol.

Hot methyl alcohol solution. Active.	Methyl alcohol insoluble residue. Inactive. Yield: 3 per cent of N. S. M.

Crystallized from cold methyl alcohol.

Cold methyl alcohol solution. Active.	Precipitate from cold methyl alcohol. Almost entirely inactive. Yield: 10 per cent of N. S. M.

Distributed between dilute methyl alcohol and petroleum ether.

Petroleum ether soluble. Active. Yield: 13 per cent of N. S. M.

Dilute methyl alcohol soluble. Inactive. Yield: 4 per cent of N. S. M.

Sterols precipitated by digitonin.

Sterol-free orange oil. Yield: 2 to 3 gm. Active.

Sterols from digitonide. Inactive.

Refluxed in hot 20 per cent alcoholic potassium hydrate.

N. S. M. contains all the active material.

Fatty acids. Inactive.

Sterols again precipitated by digitonin.

Sterol-free oil. Active.

Sterols. Inactive.

Treated with boiling methyl alcohol.

Orange solution in methyl alcohol. Active. Yield: 700 to 1000 mg. (proved active in single doses of 5 to 10 mg.).

Residue insoluble in hot methyl alcohol. Inactive. Yield: 50 to 100 mg.

Distilled in vacuo.

Fraction I	*Fraction II*	*Fraction III*
Up to 200° C. at 0.8 mm. Low acidity. 33 per cent of total.	200° to 233° C. at 0.5 mm. Highly active. 27 per cent of total.	Residue above 233° C. Highly active. 33 per cent of total.

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